SCIENTIFIC OPINION

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Safety and efficacy of a feed additive consisting of *Propionibacterium freudenreichii* DSM 33189 and *Lentilactobacillus buchneri* (formerly *Lactobacillus buchneri*) DSM 12856 for all animal species (Lactosan GmbH & Co.KG.)

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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 the safety and efficacy of an additive consisting of *Propionibacterium freudenreichii* DSM 33189 and *Lentilactobacillus buchneri* (formerly *Lactobacillus buchneri*) DSM 12856 as a technological additive for all animal species. The additive is intended to improve the production of silage at a proposed application rate of 1×10^8 colony-forming units (CFU)/kg fresh material. The bacterial species *P. freudenreichii* and *L. buchneri* are considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to assessment. As the identity of the strains has been clearly established and no acquired antimicrobial resistance determinants of concern were detected, the use of the strains as a silage additive is considered safe for livestock species, for consumers and for the environment. The additive is not irritant for eyes or skin but should be considered a respiratory sensitiser. In the absence of data, the FEEDAP Panel could not conclude on the potential of the additive to be a skin sensitiser. The additive at the proposed application rate of 1×10^8 CFU/kg fresh plant material showed the potential to improve the aerobic stability silage with dry matter content ranging from 30% to 70%.

Keywords: technological additive, silage additive, *Propionibacterium freudenreichii* DSM 33189, *Lentilactobacillus buchneri* (formerly *Lactobacillus buchneri*) DSM 12856, safety, efficacy, QPS

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

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 Lactosan GmbH & Co.KG.[2] for the authorisation of the product consisting of *Propionibacterium freudenreichii* DSM 33189 and *Lentilactobacillus buchneri* (formerly *Lactobacillus buchneri*) DSM 12856, 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 4(1) (authorisation of a feed additive or new use of a feed additive). The particulars and documents in support of the application were considered valid by EFSA as of 4 June 2021.

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 consisting of *Propionibacterium freudenreichii* DSM 33189 and *Lentilactobacillus buchneri* DSM 12856, when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

The additive is a preparation containing viable cells of *Propionibacterium freudenreichii* DSM 33189 and *Lentilactobacillus buchneri* (formerly *Lactobacillus buchneri*) DSM 12856. It has not been previously authorised as a feed additive in the European Union.

For one of the active agents, *L. buchneri* DSM 12856, EFSA delivered one opinion on the safety and efficacy as a technological additive (silage additive) for all animal species (EFSA FEEDAP Panel, 2011), and this microorganism is currently authorised in the European Union as a technological additive (1k2075).[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 *P. freudenreichii* DSM 33189 and *L. buchneri* DSM 12856 as a feed additive.

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]

[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] Lactosan GmbH & Co.KG, Industriestraße West 5, 8605 Kapfenberg, Austria.
[3] Commission Implementing Regulation (EU) No 1263/2011 of 5 December 2011 concerning the authorisation of *Lactobacillus buchneri* (DSM 16774), *Lactobacillus buchneri* (DSM 12856), *Lactobacillus paracasei* (DSM 16245), *Lactobacillus paracasei* (DSM 16773), *Lactobacillus plantarum* (DSM 12836), *Lactobacillus plantarum* (DSM 12837), *Lactobacillus brevis* (DSM 12835), *Lactobacillus rhamnosus* (NCIMB 30121), *Lactococcus lactis* (DSM 11037), *Lactococcus lactis* (NCIMB 30160), *Pediococcus acidilactici* (DSM 16243) and *Pediococcus pentosaceus* (DSM 12834) as feed additives for all animal species; OJ L 322, 06.12.2011, p. 3.
[4] FEED dossier reference: FAD-2021-0007.
[5] The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/publications/fad-2021-0007_en

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of *P. freudenreichii* DSM 33189 and *L. buchneri* DSM 12856 is in line with the principles laid down in Regulation (EC) No 429/2008[6] and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017a), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018a), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b) and Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019).

3. Assessment

The product under assessment is a preparation of viable cells of *P. freudenreichii* DSM 33189 and *L. buchneri* DSM 12856 intended for use as a technological additive (functional group: silage additives) for the improvement of the aerobic stability of silage with dry matter content ranging from 30% to 70% for all animal species.

3.1. Characterisation

3.1.1. Characterisation of the active agents

The active agents *P. freudenreichii* and *L. buchneri* were isolated from ripe cheese and silage, respectively, and are deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) with the accession numbers DSM 33189[7] and DSM 12856,[8] respectively. The active agents have not been genetically modified.

Taxonomical identification of both strains was confirmed [redacted]

The antimicrobial susceptibility of *P. freudenreichii* DSM 33189 and *L. buchneri* DSM 12856 [redacted] was determined [redacted]

Therefore, the two strains are considered to be susceptible to all the relevant antibiotics.

[redacted]

[6] 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.
[7] Technical dossier/Section II/Annex II 2_2.
[8] Technical dossier/Supplementary Information August 2021/SInf_Safe_Deposit_12856.
[9] Technical dossier/Section II/Annex II 2_5.
[10] Technical dossier/Section II/Annex II 2_3.
[11] Technical dossier/Section II/Annex II 2_8.
[12] Technical dossier/Section II/Annex II 2_7.
[13] Technical dossier/Section II/Annex II 2_9 and 2_10.

3.1.2. Characterisation of the additive

Each active agent culture is prepared separately. [redacted] to guarantee a minimum concentration of active agents of 5×10^{11} CFU/g of additive (1×10^{11} CFU *P. freudenreichii* DSM 33189/g and 4×10^{11} CFU *L. buchneri* DSM 12856 /g).

Analysis of five batches showed a mean value for *P. freudenreichii* of 1.3×10^{11} CFU/g (range 1.3×10^{11}–1.4×10^{11} CFU), for *L. buchneri* of 4.9×10^{11} CFU/g (range 4.4×10^{11}–5.4×10^{11} CFU) and for total counts of 6.2×10^{11} CFU/g (range 5.8×10^{11}–6.8×10^{11} CFU/g).[16]

A total of three batches were analysed for microbiological contamination,[17] mycotoxins[18] and heavy metals and arsenic concentrations[19]. Regarding the specifications for the microbiological contaminants, limits are set for *Enterobacteriaceae* ($< 10^3$ CFU/g), *Salmonella* spp. (no detection in 25 g), yeasts and filamentous fungi ($< 10^3$ CFU/g). Analysis of three batches of the additive showed compliance with these limits.[17] Mycotoxins (including aflatoxins (B1, B2, G1, and G2), deoxynivalenol and zearalenone), heavy metals and arsenic results were all below the respective limits of quantification.[20,21]

Three batches of the additive were analysed for bulk density, and results showed an average of 463 kg/m^3 (range 460–470 kg/m^3).[20]

The dusting potential of three batches of the additive by Stauber–Heubach method showed a mean value of 0.99 g/m^3 air (range: 0.64–1.32 g/m^3 air). The same three batches were tested for particle size distribution by laser diffraction; results showed that approximately 46% of the additive consists of particles with diameters below 100 μm, 29% below 50 μm and 10% below 10 μm.[21]

3.1.3. Stability

Three batches of the additive were tested for shelf-life when stored in aluminium-polyethylene sealed bags[22] at 20°C for 12 months.[23] Negligible losses were observed at the end of the study (< 0.5 log of the initial value).

The stability in water was studied by suspending 1 g of the additive (three batches) in 19 mL of water, maintaining such suspension at 4°C for 7 days or at 20°C for 2 days. Negligible losses were observed for both conditions tested, with < 0.5 log of the initial value.[24]

3.1.4. Conditions of use

The additive is intended to be used as a silage additive, with the aim to improve the aerobic stability of silage, in forages for all animal species. It is intended to be applied as an aqueous suspension, using a spraying device, to forages with dry matter content ranging from 30% to 70%, at a proposed minimum inclusion level of 1×10^8 CFU/kg fresh material.

3.2. Safety

3.2.1. Safety for the target species, consumers and the environment

The species *P. freudenreichii* 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, 2020a,b). This approach requires the identity of the strains to be conclusively established and evidence provided to document that the strains lack acquired determinants for resistance to antibiotics of human and veterinary importance. In the view of the FEEDAP Panel, the identity of the strains was established,

[14] Technical dossier/Section II/Annex II 3_5.
[15] Technical dossier/Section II/Annex II 3_6.
[16] Technical dossier/Section II/Annex II_1_3.
[17] Technical dossier/Section II/Annex II_1_4.
[18] Technical dossier/Section II/Annex II_1_5.
[19] Technical dossier/Section II/Annex II_1_6.
[20] Technical dossier/Supplementary Information August 2021/SInf_Density_33189_12856.
[21] Technical dossier/Section III/Annex III 3_1.
[22] Technical dossier/Supplementary Information August 2021/Accompanying_letter_33189_12856.
[23] Technical dossier/Section II/Annex II 4_1.
[24] Technical dossier/Section II/Annex II 4_2.

and the antibiotic resistance qualification met. Consequently, *P. freudenreichii* DSM 33189 and *L. buchneri* DSM 12856 are presumed safe for the target species, consumers and the environment.

3.2.2. Safety for user

The dusting potential reported (up to 1.32 g/m^3 air) indicated that exposure by inhalation is possible. Owing to the proteinaceous nature of the active agents, the additive should be considered a respiratory sensitiser.

A GLP-compliant *in vitro* skin irritation test was performed according to OECD Test Guideline 439.[25] The results of this study showed that the test item (preparation of *P. freudenreichii* DSM 33189 and *L. buchneri* DSM 12856) is not a skin irritant (No UN GHS Category).

An *in vivo* acute eye irritation/corrosion test according to OECD Test Guideline 405 and GLP compliant, using New Zealand rabbits was performed.[26] The ocular reactions observed during the study were slight to moderate and totally reversible between day one and two of the study. The results indicated that the test item (preparation of *P. freudenreichii* DSM 33189 and *L. buchneri* DSM 12856) is not irritating to rabbit eyes and does not have to be classified in accordance with CLP regulation EC No 1272/2008.

No data on the potential of the additive to be a skin sensitiser was submitted.

Once an active agent has been authorised as a silage additive, different formulations can be placed on the market with reference to that authorisation. For this specific product, the excipients used in the preparation of the final formulation are not expected to introduce additional risks.

3.2.2.1. Conclusions on safety for user

The additive is not irritant for eyes and skin but should be considered a respiratory sensitiser. No conclusions can be drawn on the potential of the additive to cause skin sensitisation.

3.3. Efficacy

Three laboratory studies were conducted with forages representing materials easy to ensile (study 1) and moderately difficult to ensile (studies 2 and 3) as specified by Regulation (EC) No 429/2008 (Table 1). All the studies included a control group and a group in which the additive was applied to the forage at a concentration of 1×10^8 CFU/kg of fresh forage. An aqueous suspension of the additive was prepared and then sprayed onto the forage prior to ensiling. In the control silos, the same volume of water was added, but without the additive. In all studies the forage was ensiled for 90 days in mini-silos (three replicates per treatment) with a capacity of 5.0 L (study 1) or 6.5 L (studies 2 and 3). All experiments were conducted at 20 $\pm$ 1°C.

Table 1: Characteristics of the forage samples used in the three ensiling experiments

Study	Test material	Dry matter content (%)	Water-soluble carbohydrate content (% fresh matter)
1[27]	Maize all plant	31.7	4.6
2[28]	Grass 2nd cut (*Festuca arundinacea*)	35.0	2.9
3[29]	Maize cob mix	65.5	2.1

After the ensiling period, the silos were opened and the contents were analysed for dry matter, pH, lactic, acetic and propionic acids, ammonia and ethanol concentrations. Aerobic stability was assessed by taking samples from each silo and exposing them to air with continuous monitoring of temperature. A rise of 3°C above room temperature was considered as indicator of silage deterioration, and the time at which that rise was observed was taken as a measure of the aerobic stability of treated and control silages. A minimum increase of stability of the treated silage of two days compared to that shown by the untreated control is considered as evidence of aerobic stability.

[25] Technical dossier/Section III/Annex III 3_2.
[26] Technical dossier/Section III/Annex III 3_3.
[27] Technical dossier/Section IV/Annex IV 3.
[28] Technical dossier/Section IV/Annex IV 1.
[29] Technical dossier/Section IV/Annex IV 2.

Data were analysed using the Mann–Whitney test and significance declared at ≤ 0.05. Results are shown in Table 2.

Table 2: Summary of the analysis of ensiled material recovered at the end of the ensiling period

Study	Application rate (CFU/kg forage)	Dry matter loss (%)	pH	Lactic acid (%)	Acetic acid (%)	Ammonia-N (% total N)	Aerobic stability (days)
1	0	1.8	3.8	1.8	0.3	6.1	2.2
	1×10^8	2.5*	3.9*	1.0*	1.6*	5.3*	> 12*
2	0	3.1	4.6	1.6	0.5	9.9	3.2
	1×10^8	3.8	4.5	0.6*	1.8*	9.3	> 11*
3	0	1.4	4.0	1.5	0.4	7.1	2.5
	1×10^8	1.4	4.2*	0.0*	2.6*	7.4	> 10.8*

CFU: colony forming unit.
*: Means in a column within a given trial are significantly different to the control $p \leq 0.05$.

The use of the additive resulted in a significant increase in the aerobic stability of silages in all three studies. As expected by the fermentation using these two species, lactic acid decreased and acetic acid increased, both significantly. In the easy to ensile plant material (study 1), a significant increase of dry matter loss and pH, and a significant decrease of ammonia-N, compared to the control, were observed. In one of the studies with moderately difficult material to ensile (study 3), pH was also significantly increased in the treated group compared to the control.

3.3.1. Conclusions on efficacy

The use of the additive has the potential to improve the aerobic stability of silages from easy and moderately difficult to ensile material having a dry matter content ranging from 30% to 70%.

4. Conclusions

Based on the QPS approach to safety assessment, both *P. freudenreichii* DSM 33189 and *L. buchneri* DSM 12856 are presumed safe for the target species, consumers and the environment.

The additive is not irritant for eyes and skin but should be considered a respiratory sensitiser. No conclusions can be drawn on skin sensitisation potential of the additive.

The additive containing a combination of *P. freudenreichii* DSM 33189 and *L. buchneri* DSM 12856 at the proposed inclusion level of 1×10^8 CFU/kg fresh plant material has the potential to improve the aerobic stability of silage from easy and moderately difficult to ensile material having a dry matter content ranging from 30% to 70%.

5. Documentation as provided to EFSA/Chronology

Date	Event
10/02/2021	Dossier received by EFSA. *P. freudenreichii* DSM 33189 and *L. buchneri* DSM 12856. Submitted by Lactosan GmbH & Co. KG
25/02/2021	Reception mandate from the European Commission
04/06/2021	Application validated by EFSA – Start of the scientific assessment
13/07/2021	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation*
13/08/2021	Reception of supplementary information from the applicant - Scientific assessment re-started
06/09/2021	Comments received from Member States
29/11/2021	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
26/01/2022	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

CFU	colony forming unit
CV	coefficient of variation

EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
MIC	minimum inhibitory concentration

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for *Propionibacterium freudenreichii* DSM 33189 and *Lactobacillus buchneri* DSM 12856

In the current application an authorisation is sought under Article 4(1) (new feed additive) for a preparation of *Propionibacterium freudenreichii* DSM 33189 and *Lactobacillus buchneri* DSM 12856 under the category/functional group 1(k) "technological additives"/"silage additives", according to Annex I of Regulation (EC) No 1831/2003. The authorisation is sought for the use of the feed additive for all animal species.

According to the Applicant, the feed additive contains non-genetically modified strains of *Propionibacterium freudenreichii* DSM 33189 and *Lactobacillus buchneri* DSM 12856 as active substances with a minimum content of 1×10^{11} and 4×10^{11} Colony Forming Units (CFU)/g feed additive, respectively.

The feed additive is intended to be added into silage through its aqueous suspension at a minimum dose of the active substances of 1×10^{8} CFU/kg fresh silage.

For the genetic identification of *Propionibacterium freudenreichii* DSM 33189 and *Lactobacillus buchneri* DSM 12856 the EURL recommends for official control the pulsed-field gel electrophoresis (PFGE), a generally recognised methodology for the genetic identification of bacterial strains.

For the enumeration of *Lactobacillus buchneri* DSM 12856 in the feed additive the EURL recommends for official control the ring-trial validated EN 15787 method.

For the enumeration of *Propionibacterium freudenreichii* DSM 33189 in the feed additive the EURL recommends for official control the pour plate method on caseine peptone, yeast extract, sodium lactate and L-cysteine agar (based on ISO 27205 standard method).

Since the unambiguous enumeration of content of *Propionibacterium freudenreichii* DSM 33189 and *Lactobacillus buchneri* DSM 12856 initially added to silage is not experimentally achievable, the EURL is not able to evaluate or recommend any method for official control for the enumeration of these microorganisms in silage.

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.