SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 23 June 2021

doi: 10.2903/j.efsa.2021.6704

Safety and efficacy of a feed additive consisting of *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) IMI 507027 for all animal species (ALL-TECHNOLOGY (IRELAND) LIMITED [Alltech Ireland])

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Mojca Fašmon Durjava, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Pier Sandro Cocconcelli, Boet Glandorf, Miguel Prieto Maradona, Maria Saarela, Rosella Brozzi, Jaume Galobart, Matteo Innocenti and Joana Revez

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 *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) IMI 507027 as a technological additive for all animal species. The additive is intended to improve the production of silage at a proposed application rate of 1×10^9 colony forming units (CFU)/kg fresh material. The bacterial species *L. plantarum* is considered by EFSA to be suitable for the qualified presumption of safety approach. As the identity of the strain has been established and no antimicrobial resistance determinants of concern were detected, the use of the strain as a silage additive is considered safe for livestock species, for consumers and for the environment. In the absence of data, the FEEDAP Panel cannot conclude on the potential of the additive to be a skin/eye irritant or a skin sensitiser. Given the proteinaceous nature of the active agent, the additive should be considered a respiratory sensitiser. The additive at the proposed application rate of 1×10^9 CFU/kg fresh material has the potential to improve the fermentation of the silages from easy to moderately difficult to ensile forages.

Keywords: technological additive, silage additive, *Lactiplantibacillus plantarum* IMI 507027, safety, efficacy, QPS

Requestor: European Commission

Question number: EFSA-Q-2020-00696

Correspondence: feedap@efsa.europa.eu

Panel members: Giovanna Azimonti, Vasileios Bampidis Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Maryline Kouba, Mojca Fašmon Durjava, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa and Ruud Woutersen.

Legal notice: Relevant information or parts of this scientific output have been blackened in accordance with the confidentiality requests formulated by the applicant pending a decision thereon by the European Commission. The full output has been shared with the European Commission, EU Member States and the applicant. The blackening will be subject to review once the decision on the confidentiality requests is adopted by the European Commission.

Declarations of interest: The declarations of interest of all scientific experts active in EFSA's work are available at https://ess.efsa.europa.eu/doi/doiweb/doisearch.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Fašmon Durjava M, Kouba M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Cocconcelli PS, Glandorf B, Maradona MP, Saarela M, Brozzi R, Galobart J, Innocenti M and Revez J, 2021. Scientific Opinion on the safety and efficacy of a feed additive consisting of *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) IMI 507027 for all animal species (ALL-TECHNOLOGY (IRELAND) LIMITED [Alltech Ireland]). EFSA Journal 2021;19(7):6704, 10 pp. https://doi.org/10.2903/j.efsa.2021.6704

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.

Table of contents

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 ALL-TECHNOLOGY (IRELAND) LIMITED [Alltech Ireland][2] for the authorisation of the product *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) IMI 507027, 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 7 January 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 *Lactiplantibacillus plantarum* IMI 507027, 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 *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) IMI 507027. It has not been previously authorised as a feed additive in the European Union.

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 *Lactiplantibacillus plantarum* IMI 507027 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.[4]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of *Lactiplantibacillus plantarum* IMI 507027 is in line with the principles laid down in Regulation (EC) No 429/2008[5] and the relevant guidance documents: Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018a), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), 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 environment (EFSA FEEDAP Panel, 2019) and Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018b).

[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] ALL-TECHNOLOGY (IRELAND) LIMITED [Alltech Ireland], Sarney, Summerhill Rd., A86X006 Dunboyne, Co. Meath, Ireland.
[3] FEED dossier reference: FAD-2020-0079.
[4] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2020-0075-0078-0079-0080-lactobacilli.pdf
[5] 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.

3. Assessment

The product under assessment is a preparation of viable cells of *Lactiplantibacillus plantarum* IMI 507027 intended for use as a technological additive (functional group: silage additives) in easy and moderately difficult to ensile forages for all animal species.

3.1. Characterisation

3.1.1. Characterisation of the active agent

The active agent was isolated from grass silage. It is deposited in the Centre for Agriculture and Bioscience International culture collection, formerly International Mycological Institute, CABI-IMI Culture collection, with the accession number IMI 507027.[6] It has not been genetically modified.

Taxonomical identification was confirmed with alignment-free genome distance estimation with Mash using MinHash and OrthoANI to calculate *in silico* average nucleotide identity (ANI) based on the whole genome sequence. Results showed that *L. plantarum* JDM1 was the closest matching NCBI RefSeq genome with a Mash distance of 0.00016 and an OrthoANI value of 99.97%. In addition, the strain *L. plantarum* IMI 507027 has a calculated OrthoANI value of 99.13% with the type strain of this species, *L. plantarum* ATCC 14917^T.[7]

The bacterial strain was tested for antibiotic susceptibility using a broth microdilution method.[8] The battery of antibiotics used included those recommended by EFSA (EFSA FEEDAP Panel, 2018a). All the minimum inhibitory concentration values were equal or fell below the corresponding cut-off values for *L. plantarum*. Therefore, the strain is considered to be susceptible to all the relevant antibiotics.

The whole genome sequence of the strain was searched for antibiotic resistance genes using the ABRicate tool with thresholds of 70% for both identity and coverage, at nucleotide and protein level.[7] The databases used were ARG-ANNOT, ecOH, MEGARes, NCBI Bacterial Antimicrobial Resistance Reference Gene Database and ResFinder. No hits of concern were identified.

3.1.2. Characterisation of the additive

The inoculum of the active agent is prepared [redacted] to guarantee a minimum concentration of active agent of 1×10^{10} CFU/g of additive.

Analysis of five batches showed a mean value of 1.1×10^{11} CFU/g (range 9.2×10^{10}–1.3×10^{11} CFU/g).[9]

A total of four batches were analysed for microbiological contamination and mycotoxins, heavy metals and arsenic concentrations.[10] Regarding the specifications for the microbiological contaminants, limits are set for total coliforms (1,000 CFU/g), β-glucuronidase-positive *Escherichia coli* (100 CFU/g), coagulase-positive staphylococci (including *Staphylococcus aureus*) (10 CFU/g), *Salmonella* spp. (no detection in 25 g), *Listeria monocytogenes* (no detection in 25 g), *Clostridium perfringens* (100 CFU/g), anaerobic sulfite reducers (100 CFU/g), yeasts (1,000 CFU/g) and filamentous fungi (1,000 CFU/g). Analysis of four batches of the additive showed compliance with these limits.[10] The same batches were tested for the following mycotoxins which were not detected: aflatoxins (B1, B2, G1, and G2), deoxynivalenol, zearalenone, ochratoxin A, fumonisins B1 + B2, T-2 and HT-2 toxins. Average values of the analyses of the heavy metals and arsenic were: 0.058 mg Pb/kg (range 0.049–0.082), 0.030 mg Hg/kg (range 0.027–0.034), 0.017 mg Cd/kg (range 0.015–0.019), and 0.049 mg As/kg (range 0.038–0.059).[11] The levels of the detected impurities do not raise concerns.

The additive has an average density of 1,321 kg/m^3 (range: 1,320–1,322 kg/m^3) and an average bulk density of 378 kg/m^3 (range: 375–380 kg/m^3). The dusting potential of the additive was

[6] Technical dossier/Section II/Annex II 2_4.
[7] Technical dossier/Section II/Annex II 2_3.
[8] Technical dossier/Section II/Annex II 2_8.
[9] Technical dossier/Section II/Annex II 1_2.
[10] Technical dossier/Section II/Annex II 1_3.
[11] Technical dossier/Section II/Annex II 1_3 with the following Limits of Detection: aflatoxins (B1, B2, G1, and G2): 1 μg/kg, deoxynivalenol 20 μg/kg, zearalenone (10 μg/kg), ochratoxin A (0.50 μg/kg), fumonisins B1 and B2 (10 μg/kg), HT-2 toxin (5.0 μg/kg), T-2 toxin (2.5 μg/kg), Pb (0.0017 mg/kg), Hg (0.0017 mg/kg), Cd (0.0017 mg/kg) and As (0.0067 mg/kg).

measured in three batches (Stauber–Heubach) and showed a mean value of 6.72 g/m^3 (range: 6.46–6.89 g/m^3).[12] The same three batches were tested for particle size distribution by laser diffraction; results showed that approximately 34% of the additive consists of particles with diameters below 100 μm, 27% below 50 μm and 15% below 10 μm.[12]

3.1.3. Stability

Four batches of the additive were tested for shelf-life by storing the additive in sealed aluminium foil bags at 4°C for 3 months,[13] at 25°C with 60% relative humidity (RH) for 2 months[14] and at 30°C with 65% RH for 3 months.[15] Negligible losses were observed at 4°C and 25°C (< 0.5 log of the initial value), while at 30°C losses were on average 0.5 log of the initial value.

The stability in water was studied by suspending 1 g of the additive (one batch) in 1 L of water and then storing for 48 h at 4°C and 20°C. Negligible losses were observed for both temperatures tested, with < 0.5 log of the initial value.[16]

3.1.4. Conditions of use

The additive is intended for use in easy and moderately difficult to ensile forages at a proposed minimum concentration of 1×10^9 CFU/kg fresh material for all animal species.[17] It is to be applied as such or as an aqueous suspension.

3.2. Safety

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

The species *L. plantarum* is considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to safety assessment (EFSA, 2007, EFSA BIOHAZ Panel, 2020). This approach requires the identity of the strain to be conclusively established and evidence that the strain lacks acquired determinants for resistance to antibiotics of human and veterinary importance. In the view of the FEEDAP Panel, the identity of the strain was established as *L. plantarum* and the antimicrobial resistance qualification has been met. Consequently, *Lactiplantibacillus plantarum* IMI 507027 is presumed safe for the target species, consumers and the environment.

3.2.2. Safety for user

No studies were submitted regarding the effects of the additive to the respiratory tract, skin or eyes.

The dusting potential reported is high (6.7 g/m^3), thus exposure by inhalation is possible. Owing to the proteinaceous nature of the active agent, the additive should be considered a 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. 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 should be considered a respiratory sensitiser. No conclusions can be drawn on the potential of the additive to cause skin and eye irritation or skin sensitisation.

3.3. Efficacy

Five laboratory studies were conducted with forages representing materials easy to ensile (studies 1–3)[18] and moderately difficult to ensile (studies 4[19] and 5[18]) as specified by Regulation (EC) No 429/2008 (Table 1). All the studies included a control and a group in which *Lactiplantibacillus plantarum*

[12] Technical dossier/Section II/Annex II 1_4.
[13] Technical dossier/Section II/Annex II 4_1.
[14] Technical dossier/Section II/Annex II 4_2.
[15] Technical dossier/Section II/Annex II 4_3.
[16] Technical dossier/Section II/Annex II 4_5.
[17] During the assessment, the applicant clarified that under the conditions of use, the minimum dose is **1×10^9 CFU/kg** (Technical dossier/Response to EFSA - FAD-2020-0079_SIn_110221).
[18] Technical dossier/Section IV/Annex IV 2.1 and Response to EFSA - FAD-2020-0079_SIn_110221.
[19] Technical dossier/Section IV/Annex IV 2.2 and Response to EFSA - FAD-2020-0079_SIn_110221.

IMI 507027 was applied to the forage at a concentration of 1×10^9 CFU/kg of fresh forage. Analytical confirmation of the counts of the two batches of the additive used for the studies was provided. 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 studies 1–3 and 5, the forage was ensiled for 90 days in mini-silos (five replicates per treatment) with a capacity of 1.75 L with the potential to vent gas. In study 4, the forage was ensiled for 100 days in mini-silos (four replicates per treatment) with a capacity of 20 L and a device to vent gas. All experiments were conducted at 20 ± 1°C.

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

Study	Test material	Dry matter content (%)	Water-soluble carbohydrate content (% fresh matter)
1	Grass-clover (2nd cut)[(a)]	40.1	5.3
2	Grass-clover (2nd cut)[(a)]	39.8	3.4
3	Grass-clover (2nd cut)[(a)]	27.1	3.6
4	Meadow grass[(b)]	26.5	2.2
5	Grass-clover (2nd cut)[(a)]	24.0	2.1

(a): Grass-clover consisting of timothy (*Phleum pratense*), perennial ryegrass (*Lolium perenne*), meadow fescue (*Festuca pratensis*), red clover (*Trifolium pratense*) and white clover (*Trifolium repens*).
(b): Meadow grass consisting of Italian ryegrass (*Lolium multiflorum*), perennial ryegrass (*Lolium perenne*), white clover (*Trifolium repens*), common dandelion (*Taraxacum officinale*), ribwort plantain (*Plantago lanceolate*) and common vetch (*Vicia sativa*).

After 90 days (or 100 days for study 4), the silos were opened and the contents were analysed for dry matter (DM), pH, lactic, acetic and propionic acids and ethanol concentrations, and ammonia. Aerobic stability was assessed at the end of each experiment by taking samples from each silo and exposing to air with continuous monitoring of temperature. A rise of 3°C above room temperature was considered as an 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.

Data were analysed using the Wilcoxon signed-rank test (studies 1–3 and 5) or Mann–Whitney test (study 4) and significance declared at $p < 0.05$. Results are shown in Table 2.

Table 2: Summary of the analysis of ensiled material recovered at the end of the ensiling period with *Lactiplantibacillus plantarum* IMI 507027

Study	Application rate (CFU/kg forage)	Dry matter (DM) loss (%)	pH	Lactic acid (%)[(a)]	Acetic acid (%)[(a)]	Ammonia-N (% total N)	Aerobic stability (h)
1	0	0.9	5.10	2.1	0.3	4.5	171
	1×10^9	0.7	3.96*	4.7*	0.4	1.3*	277
2	0	0.9	4.81	2.6	0.6	6.6	230
	1×10^9	0.7	4.06*	4.5*	0.7	3.0*	287*
3	0	1.1	4.43	2.8	0.4	10.1	207
	1×10^9	0.5*	3.82*	4.3*	0.3	2.0*	208
4	0	2.8	3.89	7.3	1.3	6.4	57
	1×10^9	2.4	3.87*	8.0	1.5*	6.1	102*
5	0	0.8	4.25	2.9	0.5	9.4	218
	1×10^9	0.5*	3.99*	3.4	0.5	5.0*	226

CFU: colony forming unit; DM: dry matter.
(a): Expressed as percentage in silage juice for studies 1, 2, 3 and 5 and in percentage of dry matter for study 4.
*: Means in a column within a given trial are significantly different to the control $p < 0.05$.

The addition of the additive resulted in a significant reduction in pH in all studies (although only a marginal difference was observed in study 4) and a reduction in ammonia-N production in all studies with easy to ensile and in one study with moderately difficult to ensile material. However, dry matter loss was significantly reduced only in two of these studies. An increase of lactic acid was observed in

the three studies with easy to ensile material, while acetic acid increased in one study with moderately difficult to ensile material. Regarding the aerobic stability, a positive outcome was observed only in studies 2 and 4, but reaching a 48 h difference only in study 2.

3.3.1. Conclusions on efficacy

Considering the effects on ammonia-N it can be concluded that the *Lactiplantibacillus plantarum* IMI 507027 at the proposed inclusion rate has the potential to improve the preservation of nutrients in silage prepared with easy and moderately difficult to ensile material.

4. Conclusions

Based on the QPS approach to safety assessment, *Lactiplantibacillus plantarum* IMI 507027 is presumed safe for the target species, consumers and the environment.

The additive should be considered a respiratory sensitiser. No conclusions can be drawn on the eye and skin irritancy, or skin sensitisation potential of the additive.

Lactiplantibacillus plantarum IMI 507027 at a concentration of 1×10^9 CFU/kg plant material has the potential to improve the preservation of nutrients in silage prepared with easy and moderately difficult to ensile material.

5. Documentation as provided to EFSA/Chronology

Date	Event
14/10/2020	Dossier received by EFSA. *L. plantarum* IMI 507027. Submitted by ALL-TECHNOLOGY (IRELAND) LIMITED [Alltech Ireland]
19/10/2020	Reception mandate from the European Commission
07/01/2021	Application validated by EFSA – Start of the scientific assessment
11/02/2021	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 and efficacy*
08/03/2021	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
07/04/2021	Reception of supplementary information from the applicant - Scientific assessment re-started
09/04/2021	Comments received from Member States
23/06/2021	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://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2007.587

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Hilbert F, Lindqvist R, Nauta M, Peixe L, Ru G, Simmons M, Skandamis P, Suffredini E, Cocconcelli PS, Fernández Escámez PS, Maradona MP, Querol A, Suarez JE, Sundh I, Vlak J, Barizzone F, Correia S and Herman L, 2020. Scientific Opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA (2017–2019). EFSA Journal 2020;18(2):5966, 56 pp. https://doi.org/10.2903/j.efsa.2020.5966

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

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, 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, Wester P, Anguita M, Galobart J and Innocenti ML, 2017a. Guidance on the identity, characterisation and conditions of use of feed additives. EFSA Journal 2017a;15(10):5023, 12 pp. https://doi.org/10.2903/j.efsa.2017.5023

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, 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, Wester P, Anguita M, Galobart J, Innocenti ML and Martino L, 2017b. Guidance on the assessment of the safety of feed additives for the target species. EFSA Journal 2017b;15(10):5021, 19 pp. https://doi.org/10.2903/j.efsa.2017.5021

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, 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, Wester P, Glandorf B, Herman L, Kärenlampi S, Aguilera J, Anguita M, Brozzi R and Galobart J, 2018a. Guidance on the characterisation of microorganisms used as feed additives or as production organisms. EFSA Journal 2018;16(3):5206, 24 pp. https://doi.org/10.2903/j.efsa.2018.5206

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, 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, Wester P, Anguita M, Galobart J, Innocenti ML and Martino L, 2018b. Guidance on the assessment of the efficacy of feed additives. EFSA Journal 2018;16(5):5274, 25 pp. https://doi.org/10.2903/j.efsa.2018.5274

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Bastos ML, Christensen H, Dusemund B, Kouba M, Kos Durjava M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Brock T, Knecht J, Kolar B, Beelen P, Padovani L, Tarrés-Call J, Vettori MV and Azimonti G, 2019. Guidance on the assessment of the safety of feed additives for the environment. EFSA Journal 2019;17(4):5648, 78 pp. https://doi.org/10.2903/j.efsa.2019.5648

Abbreviations

ANI	average nucleotide identity
CFU	colony forming unit
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
PFGE	pulsed-field gel electrophoresis
QPS	qualified presumption of safety
RH	relative humidity

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for[20] *Lactobacillus plantarum* IMI 507027

In the current applications authorisations are sought under Article 4(1) for *Lactobacillus rhamnosus* IMI 507023, *Lactobacillus plantarum* IMI 507026, *Lactobacillus plantarum* IMI 507027 and *Lactobacillus plantarum* IMI 507028 under the category/functional group 1(k) "technological additives"/ "silage additives", according to Annex I of Regulation (EC) No 1831/2003. The authorisations are sought for the use of the *feed additives* for all animal species.

According to the Applicant, the feed additives contain as *active substance* viable cells *of Lactobacillus rhamnosus* IMI 507023, *Lactobacillus plantarum* IMI 507026, *Lactobacillus plantarum* IMI 507027 and *Lactobacillus plantarum* IMI 507028, respectively. The feed additives are to be marketed as preparations containing a minimum content of 1×10^{10} Colony Forming Units (CFU) of *Lactobacillus rhamnosus* IMI 507023 or *Lactobacillus plantarum* IMI 507026 or *Lactobacillus plantarum* IMI 507027 *or Lactobacillus plantarum* IMI 507028/g in the respective feed additive. The *feed additives* are intended to be used at a minimum dose of 1×10^6 CFU/kg[17] fresh *silage*.

For the identification of *Lactobacillus rhamnosus* IMI 507023, *Lactobacillus plantarum* IMI 507026, *Lactobacillus plantarum* IMI 507027 and *Lactobacillus plantarum* IMI 507028 the EURL recommends for official control Pulsed-Field Gel Electrophoresis (PFGE), a recognised methodology for the genetic identification of bacterial strains.

For the enumeration of *Lactobacillus rhamnosus* IMI 507023, *Lactobacillus plantarum* IMI 507026, *Lactobacillus plantarum* IMI 507027 and *Lactobacillus plantarum* IMI 507028 in the feed additives the EURL recommends for official control the ring-trial validated spread plate method EN 15787.

Since the unambiguous determination of the content of *Lactobacillus rhamnosus* IMI 507023 or *Lactobacillus plantarum* IMI 507026 or *Lactobacillus plantarum* IMI 507027 or *Lactobacillus plantarum* IMI 507028 initially added to silage is not experimentally achievable, the EURL is not able to evaluate or recommend any method for official control for the determination *Lactobacillus rhamnosus* IMI 507023, *Lactobacillus plantarum* IMI 507026, *Lactobacillus plantarum* IMI 507027 and *Lactobacillus plantarum* IMI 507028 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.

[20] The EURL produced a combined report for *Lactobacillus rhamnosus* IMI 507023, *Lactobacillus plantarum* IMI 507026, *Lactobacillus plantarum* IMI 507027 and *Lactobacillus plantarum* IMI 507028.