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Safety and efficacy of a feed additive consisting of *Lacticaseibacillus rhamnosus* (formerly *Lactobacillus rhamnosus*) IMI 507023 for all animal species (ALL-TECHNOLOGY (IRELAND) LIMITED [Alltech Ireland])

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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 *Lacticaseibacillus rhamnosus* (formerly *Lactobacillus rhamnosus*) IMI 507023 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. rhamnosus* 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 acquired 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.

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Keywords: technological additive, silage additive, *Lacticaseibacillus rhamnosus* IMI 507023, safety, efficacy, QPS

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1. Introduction

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

Regulation (EC) No 1831/2003¹ 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 *Lacticaseibacillus rhamnosus* (formerly *Lactobacillus rhamnosus*) IMI 507023, 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 *Lacticaseibacillus rhamnosus* IMI 507023, 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 *Lacticaseibacillus rhamnosus* (formerly *Lactobacillus rhamnosus*) IMI 507023. 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 dossier³ in support of the authorisation request for the use of *Lacticaseibacillus rhamnosus* IMI 507023 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 *Lacticaseibacillus rhamnosus* IMI 507023 is in line with the principles laid down in Regulation (EC) No 429/2008⁵ 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., A86 × 006 Dunboyne, Co. Meath, Ireland.

³ FEED dossier reference: FAD-2020-0075.

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 *Lacticaseibacillus rhamnosus* IMI 507023 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 maize 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 507023.⁶ It has not been genetically modified.

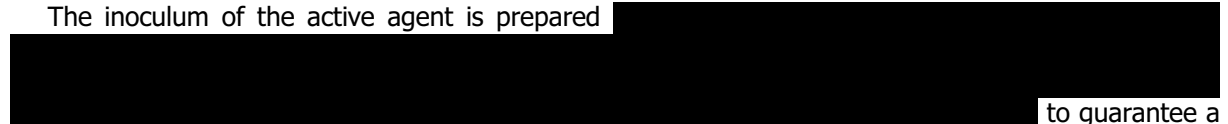
Taxonomical identification was confirmed with alignment-free genome distance estimation with Mash using MinHash and OrthoANI to calculate *in silico* average nucleotide identity (ANI) based on the whole genome sequence. Results showed that the strain *L. rhamnosus* IMI 507023 had a Mash distance of 0.001041 and a calculated OrthoANI value of 99.98% with *L. rhamnosus* DSM 20021^{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 (MIC) values were equal or fell below the corresponding cut-off values for *L. rhamnosus*, except for chloramphenicol (MIC: 8 mg/L vs cut-off value: 4 mg/L). Exceeding the cut-off value by one dilution is considered to be within the normal range of variation and thus, not a matter of concern. 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

 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 1.3×10^{11} CFU/g (range 9.3×10^{10} – 1.6×10^{11} CFU/g).⁹

A total of four batches were analysed for microbiological contamination and mycotoxins and 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, HT-2 toxin and T-2 toxin. Average values of the analyses of the heavy metals and arsenic were: 0.051 mg Pb/kg (range 0.045–0.058), 0.0052 mg Hg/kg (up to 0.0133) and 0.0276 mg As/kg (range 0.02–0.04). Cadmium was not detected.¹¹ The levels of the detected impurities do not raise concerns.

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 1_2.

¹⁰ Technical dossier/Section II/Annex 1_3.

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

The additive has an average density of 1,507 kg/m³ (range: 1,507–1,508 kg/m³) and an average bulk density of 318 kg/m³ (range: 318–320 kg/m³). The dusting potential of the additive was measured in three batches (Stauber–Heubach) and showed a mean value of 5.99 g/m³ air (range: 5.63–6.50 g/m³ air).¹² The same three batches were tested for particle size distribution by laser diffraction; results showed that ~ 66% of the additive consists of particles with diameters below 100 µm, 59% below 50 µm and 24% below 10 µm.¹²

3.1.3. Stability

Four batches of the additive were tested for shelf-life by storing in sealed aluminium foil bags at 4°C for 3 months,¹³ at 25°C with 60% relative humidity (RH) for 2 months¹⁴ 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 0.6 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 inclusion level of 1 × 10⁹ 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. rhamnosus* 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. rhamnosus* and the antibiotic resistance qualification has been met. Consequently, *Lacticaseibacillus rhamnosus* IMI 507023 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 (5.99 g/m³), 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.

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_4.

¹⁷ Technical dossier/FAD-2020-0075_AppSIn_060421.

3.3. Efficacy

Nine laboratory studies were conducted with forages representing materials easy to ensile (studies 1,¹⁸ 2,¹⁸ 3¹⁸ and 4¹⁹) and moderately difficult to ensile (studies 5,¹⁹ 6,¹⁹ 7,²⁰ 8¹⁸ and 9¹⁹) as specified by Regulation (EC) No 429/2008 (Table 1). All the studies included a control group and a group in which *Lacticaseibacillus rhamnosus* IMI 507023 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 all studies except study 7, the forage was ensiled for 90 days in mini-silos (five replicates per treatment) with a capacity of 1.75 L with potential to vent gas. In study 7, 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 $20 \pm 1^\circ\text{C}$.

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

| Study | Test material | Dry matter content (%) | Water-soluble carbohydrate content (% fresh matter) |
|-------|---|------------------------|---|
| 1 | Grass-clover (2nd cut) ^(a) | 40.1 | 5.27 |
| 2 | Grass-clover (2nd cut) ^(a) | 39.8 | 3.44 |
| 3 | Grass-clover (2nd cut) ^(a) | 27.1 | 3.57 |
| 4 | Grass-clover (3rd/4th cut) ^(a) | 38.8 | 3.96 |
| 5 | Grass-clover (3rd/4th cut) ^(a) | 38.6 | 2.83 |
| 6 | Grass-clover (3rd/4th cut) ^(a) | 27.6 | 2.84 |
| 7 | Meadow grass ^(b) | 26.5 | 2.16 |
| 8 | Grass-clover (2nd cut) ^(a) | 24.0 | 2.09 |
| 9 | Grass-clover (3rd/4th cut) ^(a) | 25.0 | 1.78 |

(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): Permanent meadow grass consisting of Italian ryegrass (*Lolium multiflorum*), perennial ryegrass (*Lolium perenne*), white clover (*Trifolium repens*), common dandelion (*Taraxacum officinale*), ribwort plantain (*Plantago lanceolata*) and common vetch (*Vicia sativa*).

After the ensiling period, the silos were opened and the contents were analysed for dry matter (DM), pH, lactic, acetic and propionic acids, ammonia and ethanol concentrations. 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 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 2 days compared to that shown by the untreated control is considered as evidence of aerobic stability.

Data were analysed using the non-parametric Wilcoxon signed-rank test (all studies except study 7 which applied Mann–Whitney test) and significance declared at $p < 0.05$. Results are shown in Table 2.

The addition of the additive resulted in a significant reduction of dry matter loss in all studies except study 7, reduction in pH in all studies (despite the marginal difference in study 7), increase of lactic acid concentration in all studies with easy to ensile forage and in two moderately difficult to ensile forages, and a reduction in ammonia-N production in all easy to ensile forages and in four out of five moderately difficult to ensile forages. Regarding the aerobic stability, a positive outcome was observed only in study 7, but the difference was below the 48h threshold.

¹⁸ Technical dossier/Section IV/Annex IV 2.1 and Supplementary Information April 2021.

¹⁹ Technical dossier/Section IV/Annex IV 2.2 and Supplementary Information April 2021.

²⁰ Technical dossier/Section IV/Annex IV 2.3 and Supplementary Information April 2021.

Table 2: Summary of the analysis of ensiled material recovered at the end of the ensiling period with *Lacticaseibacillus rhamnosus* IMI 507023

| 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 | 0.89 | 5.10 | 2.05 | 0.33 | 4.47 | 171 |
| | 1 × 10 ⁹ | 0.47* | 3.79* | 5.08* | 0.19 | 1.62* | 242 |
| 2 | 0 | 0.91 | 4.81 | 2.62 | 0.60 | 6.59 | 230 |
| | 1 × 10 ⁹ | 0.45* | 3.87* | 4.75* | 0.18* | 2.37* | 234 |
| 3 | 0 | 1.13 | 4.43 | 2.77 | 0.41 | 10.11 | 207 |
| | 1 × 10 ⁹ | 0.48* | 3.68* | 4.86* | 0.18 | 3.50* | 201 |
| 4 | 0 | 0.80 | 4.85 | 2.23 | 0.39 | 5.72 | 224 |
| | 1 × 10 ⁹ | 0.55* | 3.96* | 4.44* | 0.20* | 3.77* | 248 |
| 5 | 0 | 0.87 | 4.49 | 3.41 | 0.57 | 6.09 | 245 |
| | 1 × 10 ⁹ | 0.55* | 4.03* | 4.30 | 0.25* | 4.38* | 234 |
| 6 | 0 | 0.99 | 4.39 | 2.89 | 0.53 | 10.14 | 243 |
| | 1 × 10 ⁹ | 0.47* | 3.88* | 4.35* | 0.28* | 6.51* | 220 |
| 7 | 0 | 2.79 | 3.89 | 7.29 | 1.28 | 6.44 | 57 |
| | 1 × 10 ⁹ | 2.64 | 3.88* | 7.49 | 1.35 | 6.45 | 99* |
| 8 | 0 | 0.75 | 4.25 | 2.91 | 0.45 | 9.37 | 218 |
| | 1 × 10 ⁹ | 0.47* | 3.92* | 3.62* | 0.31 | 6.05* | 206 |
| 9 | 0 | 1.30 | 4.42 | 2.59 | 0.52 | 13.19 | 258 |
| | 1 × 10 ⁹ | 0.69* | 4.09* | 3.49 | 0.50 | 9.63* | 218 |

CFU: colony forming unit; DM: dry matter.

(a): Expressed as percentage in silage juice for all studies except for study 7 that is expressed in percentage of dry matter.

*: Means in a column within a given trial are significantly different to the control $p < 0.05$.

3.3.1. Conclusions on efficacy

Considering the effects on dry matter loss and ammonia-N as percentage of total N, it can be concluded that the additive 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, *Lacticaseibacillus rhamnosus* IMI 507023 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.

Lacticaseibacillus rhamnosus IMI 507023 at a concentration of 1 × 10⁹ CFU/kg plant material has the potential to improve the preservation of nutrients in silage prepared with easy and moderately difficult to ensile forage material.

5. Documentation as provided to EFSA/Chronology

| Date | Event |
|------------|--|
| 14/10/2020 | Dossier received by EFSA. <i>Lacticaseibacillus rhamnosus</i> IMI 507023. 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. <i>Issues: characterisation and efficacy</i> |
| 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 |

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Abbreviations

| | |
|--------|--|
| 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 |

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for²¹ *L. rhamnosus* IMI 507023

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/kg²² 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.

²¹ The EURL produced a combined report for *Lactobacillus rhamnosus* IMI 507023, *Lactobacillus plantarum* IMI 507026, *Lactobacillus plantarum* IMI 507027 and *Lactobacillus plantarum* IMI 507028.

²² During the assessment, the applicant clarified that under the conditions of use, the minimum dose is 1×10^9 CFU/kg (Technical dossier/FAD-2020-0075_AppSIIn_060421).