SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 23 June 2021

doi: 10.2903/j.efsa.2021.6702

Safety and efficacy of a feed additive consisting of *Pediococcus pentosaceus* IMI 507025 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 *Pediococcus pentosaceus* IMI 507025 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 *P. pentosaceus* is considered by the European Food Safety Authority 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, *Pediococcus pentosaceus* IMI 507025, safety, efficacy, QPS

Requestor: European Commission

Question number: EFSA-Q-2020-00694

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.

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.

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.

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 *Pediococcus pentosaceus* IMI 507025 for all animal species (ALL-TECHNOLOGY (IRELAND) LIMITED [Alltech Ireland]). EFSA Journal 2021;19(7):6702, 10 pp. https://doi.org/10.2903/j.efsa.2021.6702

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 *Pediococcus pentosaceus* IMI 507025, 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 *Pediococcus pentosaceus* IMI 507025, 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 *Pediococcus pentosaceus* IMI 507025. 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 *Pediococcus pentosaceus* IMI 507025 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 *Pediococcus pentosaceus* IMI 507025 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, 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-2020-0077.
[4] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2020-0076_0077ped_pentosaceus.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 *Pediococcus pentosaceus* IMI 507025 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 originally isolated from pickled cucumbers. 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 507025.[6] It has not been genetically modified.

The 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 *P. pentosaceus* CGMCC 7049 was the closest matching NCBI RefSeq genome with a Mash distance of 0.00671909 and an OrthoANI value of 99.6%. In addition, the *P. pentosaceus* IMI 507025 had a calculated OrthoANI value of 98.9% with *P. pentosaceus* ATCC 25745.[7]

The bacterial strain was tested for antibiotic susceptibility using a broth microdilution method.[8] The 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 *Pediococcus* spp., except for kanamycin (MIC: 128 mg/L vs cut-off value: 64 mg/L), chloramphenicol (MIC: 8 mg/L vs cut-off value: 4 mg/L) and tetracycline (MIC: 64 mg/L vs cut-off value: 8 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 except for tetracycline.

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, MEGARes, NCBI Bacterial Antimicrobial Resistance Reference Gene Database and ResFinder. No hits of concern were identified.

No resistance determinants were found to justify the tetracycline resistance, thus the Panel considers that it is unlikely that this resistance may raise safety concerns.

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 1.3×10^{11} CFU/g (range $1.1 - 1.4 \times 10^{11}$ CFU/g).[9]

A total of four batches were analysed for microbiological contamination and mycotoxins, heavy metals and arsenic concentrations. 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. The same batches of the additive were tested for aflatoxins (B1, B2, G1, and G2), ochratoxin A, fumonisins B1 + B2, HT-2 toxin, T-2 toxin, deoxynivalenol, zearalenone, lead, mercury, cadmium and arsenic; results showed levels below the respective limits of detection, except for arsenic (average 0.060 mg/kg, range 0.033–0.078 mg/kg), cadmium (average in three batches where was detected 0.009 mg/kg, range 0.008–0.011 mg/kg),

[6] Technical dossier/Section II/Annex II 2_2.
[7] Technical dossier/Section II/Annex II 2_1.
[8] Technical dossier/Section II/Annex II 2_6.
[9] Technical dossier/Section II/Annex II 1_2.

mercury (average 0.015 mg/kg, range 0.011–0.019 mg/kg) and lead (average 0.035 mg/kg, range 0.021–0.054 mg/kg).[10,11] The levels of the detected impurities do not raise concerns.

The additive has an average density of 1,305 kg/m^3 (range: 1,304–1,305 kg/m^3) and an average bulk density of 314 kg/m^3 (range: 312–315 kg/m^3).[12] The dusting potential of the additive was measured in three batches (Stauber–Heubach) and showed a mean value of 3.2 g/m^3 (range: 2.9–3.7 g/m^3).[12] The same three batches were tested for particle size distribution by laser diffraction; results showed that 57% of the additive consists of particles with diameters below 100 μm, 47% 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 in sealed aluminium foil bags at 4°C for 3 months,[13] at 25°C at 60% relative humidity (RH) for 2 months[14] and at 30°C at 65% RH for 3 months.[15] Negligible losses were observed under the aforementioned conditions (< 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 before storage for 48 h at 4°C and 20°C. Negligible losses (< 0.5 log of the initial value) were observed at 4°C, whereas losses of ≥ 0.5 log were observed at 20°C.[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 1×10^9 CFU/kg fresh material for all animal species. 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 *P. pentosaceus* 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 *P. pentosaceus* and the antibiotic resistance qualification has been met. Consequently, *Pediococcus pentosaceus* IMI 507025 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 (on average 3.2 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.

[10] Technical dossier/Section II/Annex II 1_3.
[11] Limit of detection: aflatoxins (B1, B2, G1, and G2): 0.5 μg/kg, deoxynivalenol 20 μg/kg, zearalenone (10 μg/kg), ochratoxin A 0.5 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).
[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.

3.3. Efficacy

Five laboratory studies were conducted with different forages representing materials categories easy to ensile (study 1)[17] and moderately difficult to ensile (study 2,[17] 3,[17] 4[18] and 5[17]), as specified by Regulation (EC) No 429/2008 (Table 1). All the studies included a control group and a group in which *Pediococcus pentosaceus* IMI 507025 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 (3rd/4th cut)[(a)]	38.8	4.0
2	Grass-clover (3rd/4th cut)[(a)]	38.6	2.8
3	Grass-clover (3rd/4th cut)[(a)]	27.6	2.8
4	Meadow grass[(b)]	26.5	2.2
5	Grass-clover (3rd/4th cut)[(a)]	25.0	1.8

(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 non-parametric Wilcoxon signed-rank test (studies 1–3 and 5) or Mann–Whitney test (study 4)[19] and significance for all studies was 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 *Pediococcus pentosaceus* IMI 507025

Study	Application rate (CFU/kg forage)	Dry matter (DM) loss (%)	pH	Lactic acid (%)[(a)]	Acetic acid (%)[(a)]	Ammonia-N (% of total N)	Aerobic stability (h)
1	0	0.8	4.85	2.2	0.4	5.7	224
	1×10^9	0.6*	4.19*	3.4*	0.2*	4.3*	117
2	0	0.9	4.49	3.4	0.6	6.1	245
	1×10^9	0.5*	4.19*	3.7*	0.2*	4.7*	167
3	0	1.0	4.39	2.9	0.5	10.1	243
	1×10^9	0.4*	4.04*	3.1	0.1*	5.8*	117*
4	0	2.8	3.89	7.3	1.3	6.4	57
	1×10^9	2.7	3.87*	6.8	1.1*	5.9	95*

[17] Technical dossier/Section IV/Annexes IV 2_1 and Supplementary Information April 2021.
[18] Technical dossier/Section IV/Annexes IV 2_2 and Supplementary Information April 2021.
[19] Technical dossier/Supplementary Information April 2021.

Study	Application rate (CFU/kg forage)	Dry matter (DM) loss (%)	pH	Lactic acid (%)[(a)]	Acetic acid (%)[(a)]	Ammonia-N (% of total N)	Aerobic stability (h)
5	0	1.3	4.42	2.6	0.5	13.2	258
	1×10^9	0.4*	4.03*	3.2	0.3*	6.7*	150*

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 caused a significant reduction of dry matter loss and ammonia production (four out of five studies). The pH was significantly reduced in all studies (although only a marginal difference was observed in study 4). In addition, in two studies (1 and 2) a significant increase of lactic acid was seen in the inoculated silage. Regarding the aerobic stability only in study 4 a positive outcome was observed but the difference was below the 48 h threshold.

3.3.1. Conclusions on efficacy

The use of *Pediococcus pentosaceus* IMI 507025 at the proposed inclusion rate in the ensiling process 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, *Pediococcus pentosaceus* IMI 507025 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.

Pediococcus pentosaceus IMI 507025 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. *Pediococcus pentosaceus* IMI 507024. Submitted by ALL-TECHNOLOGY (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, efficacy*
09/04/2021	Comments received from Member States
16/03/2021	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
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;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 2017;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 2017;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
CV	coefficient of variation
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 for[20] *Pediococcus pentosaceus* IMI 507025

In the current applications authorisations are sought under Article 4(1) for *Pediococcus pentosaceus* IMI 507024 (FAD 2020-0076) and for *Pediococcus pentosaceus* IMI 507025 (FAD 2020-0077) under the category/functional group 1(k) "technological additives"/"silage additives", according to Annex I of Regulation (EC) No 1831/2003. Specifically, the authorisations are sought for the use of the *feed additives* in *silage* for all animal species.

According to the Applicant, the *feed additives* contain as active substance viable cells of the strains *Pediococcus pentosaceus* IMI 507024 or *Pediococcus pentosaceus* IMI 507025. The *feed additives* are to be marketed as preparations with a minimum content of $1x10^{10}$ Colony Forming Units (CFU) of *Pediococcus pentosaceus* IMI 507024 or *Pediococcus pentosaceus* IMI 507025/g *feed additive*. Both *feed additives* are intended to be used at a minimum dose of 1×10^6 CFU/kg fresh *silage*.[21]

For the identification of *Pediococcus pentosaceus* IMI 507024 and *Pediococcus pentosaceus* IMI 507025, the EURL recommends for official control Pulsed-Field Gel Electrophoresis (PFGE), a generally recognised methodology for the genetic identification of bacterial strains.

For the enumeration of *Pediococcus pentosaceus* IMI 507024 and *Pediococcus pentosaceus* IMI 507025 in the feed additives, the EURL recommends for official control the ring-trial validated spread plate method EN 15786.

Since the unambiguous determination of the content of *Pediococcus pentosaceus* IMI 507024 or *Pediococcus pentosaceus* IMI 507025 initially added to silage is not achievable by analysis, the EURL cannot evaluate nor recommend any method for official control for the determination of *Pediococcus pentosaceus* IMI 507024 or *Pediococcus pentosaceus* IMI 507025 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 *Pediococcus pentosaceus* IMI 507024 and *Pediococcus pentosaceus* IMI 507025

[21] 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-2020-0077_SIn_110221).