



EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate F – Health and Food
Food and Feed Compliance



JRC F.5/CvH/SB/AS/Ares

**Evaluation Report on the Analytical Methods submitted
in connection with the Application for Authorisation of a
Feed Additive according to Regulation (EC) No 1831/2003**

Lactiplantibacillus plantarum DSM 34271
(FEED-2023-13173; CRL/230008)



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in connection with the Application for Authorisation of a
Feed Additive according to Regulation (EC) No 1831/2003**

Dossier related to: **FEED-2023-13173 - CRL/230008**

Name of Product : ***Lactiplantibacillus plantarum DSM 34271***

Active Agent (s): **Lactiplantibacillus plantarum DSM 34271**

Rapporteur Laboratory: **European Union Reference Laboratory for
Feed Additives (EURL-FA)
JRC Geel, Belgium**

Report prepared by: **Stefano Bellorini**

Report checked by: **María José González de la Huebra**
Date: **09/11/2023**

Report approved by: **Christoph von Holst**
Date: **09/11/2023**

EXECUTIVE SUMMARY

In the current application an authorisation is sought under Article 4 for *Lactiplantibacillus plantarum* DSM 34271 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 the non-genetically modified *Lactiplantibacillus plantarum* DSM 34271 as *active substance* at a minimum concentration of 4×10^{11} Colony Forming Unit (CFU) / g. The *feed additive* is intended to be added to the forage used for ensiling process at a recommended dosage of 1×10^5 CFU / g fresh *silage*.

For the enumeration of *Lactiplantibacillus plantarum* DSM 34271 in the *feed additive* and in *silage* the Applicant proposed the ring-trial validated EN 15787 method. As the unambiguous determination of *Lactiplantibacillus plantarum* DSM 34271 added to *silage* is not achievable by analysis, the EURL cannot evaluate nor recommend any method for official control. Based on the performance characteristics, as already concluded for similar *Lactiplantibacilli* reports, the EURL recommends for official control the ring-trial validated EN 15787 method for the enumeration of *Lactiplantibacillus plantarum* DSM 34271 in the *feed additive*.

For the identification of *Lactiplantibacillus plantarum* DSM 34271, the Applicant applied a DNA sequencing method such as Whole Genome Sequencing (WGS). In former reports for similar dossiers, the EURL recommended for official control DNA sequencing methods or Pulsed-Field Gel Electrophoresis (PFGE) described in CEN Technical Specification (CEN/TS 17697). The EURL considers that all the above-mentioned methodologies are suitable for official control for the bacterial identification of *Lactiplantibacillus plantarum* DSM 34271 at strain level.

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.

KEYWORDS

Lactiplantibacillus plantarum DSM 34271, technological additives, silage additives, all animal species.

1. BACKGROUND

In the current application an authorisation is sought under Article 4(1) (new feed additive) for *Lactiplantibacillus plantarum* DSM 34271 under the category / functional group 1(k) 'technological additives' / 'silage additives', according to Annex I of Regulation (EC) No 1831/2003 [1-2]. The authorisation is sought for the use of the *feed additive* for all animal species [1-2].

According to the Applicant, the *feed additive* contains *Lactiplantibacillus plantarum* DSM 34271 as an *active substance* at a minimum concentration of 4×10^{11} Colony Forming Unit (CFU) / g [3].

The Applicant stated that the *Lactiplantibacillus plantarum* DSM 34271 is a non-genetically modified strain. The microorganism is deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) [4].

The *feed additive* is intended to be added to, easy or moderately difficult to ensile, forage at a recommended dosage of 1×10^5 CFU / g fresh *silage* [5].

Note: The EURL has previously evaluated the analytical methods for the determination of *Lactiplantibacillus plantarum* strain in the frame of previous dossiers [6].

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761, on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the duties and the tasks of the European Union Reference Laboratory concerning applications for authorisations of feed additives, the EURL is requested to submit a full evaluation report to the European Food Safety Authority for each application or group of applications. For this particular dossier, the methods of analysis submitted in connection with *Lactiplantibacillus plantarum* DSM 34271 and their suitability to be used for official controls in the frame of the authorisation were evaluated.

3. EVALUATION

Description of the analytical methods for the determination of the active substance in the feed additive, premixtures, compound feed and when appropriate water (section 2.6.1 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

For the enumeration of *Lactiplantibacillus plantarum* DSM 34271 in the *feed additive* and in *silage* the Applicant proposed the ring-trial validated EN 15787:2009 method [7]. However, the EURL is aware that this method has been revised by CEN resulting in updated method

dedicated for the enumeration of *lactobacilli* spp. in *feedingstuffs* (additives, *premixtures* and compound feeds excluding mineral feeds) that contain lactobacilli as a single microorganism component or in a mixture with other microorganisms (EN 15787:2021) [8].

Following the updated method's protocol, the sample (5 to 50 g) is suspended in phosphate buffered saline containing Polysorbate 80 (Tween® 80) (tPBS). For serial dilutions, the peptone salt solution (PSS) is used. The appropriate dilutions are then mixed on Petri plates using spread plate (or pour plate) methods with MRS (de Man, Rogosa, Sharp) agar. Alternatively, the MRS agar can be acidified or include triphenyl tetrazolium chloride (TTC). However, for routine purposes the non-modified MRS agar is an appropriate medium. The agar plates are incubated anaerobically at 37 °C for 48 to 72 h.

The following performance characteristics, expressed in terms of precision, are reported in the frame of the ring-trial validation studies after logarithmic transformation of the CFU values ranging from 7.40 to 8.03 log₁₀ CFU / g: a standard deviation for *repeatability* (S_r) ranging from 0.10 to 0.26 log₁₀ CFU / g and a standard deviation for *reproducibility* (S_R) ranging from 0.18 to 0.39 log₁₀ CFU / g [8].

In addition, a limit of quantification (LOQ) of 3 x 10³ CFU / g can be derived using the considerations of EN ISO 7218 standard [9].

Based on the available information, the EURL recommends for official control the ring-trial validated EN 15787 method for the enumeration of *Lactiplantibacillus plantarum* DSM 34271 in the *feed additive*.

As the unambiguous determination of *Lactiplantibacillus plantarum* DSM 34271 added to *silage* is not achievable by analysis, the EURL cannot evaluate nor recommend the EN 15787 or any other method for official control to enumerate *Lactiplantibacillus plantarum* DSM 34271 in *silage*.

Methods of analysis for the determination of the residues of the additive in food (section 2.6.2 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

An evaluation of corresponding methods of analysis is not relevant for the present application.

Identification/Characterisation of the feed additive (section 2.6.3 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

For the taxonomic identification of *Lactiplantibacillus plantarum* DSM 34271, the Applicant applied a DNA sequencing method such as Whole Genome Sequencing (WGS) [7].

In former reports for similar dossiers, the EURL recommended for official control DNA sequencing methods or Pulsed-Field Gel Electrophoresis (PFGE), a generally recognised methodology for the genetic identification of bacterial strains. The method has been ring-trial validated and recently published as a CEN Technical Specification CEN/TS 17697 [6,10].

The EURL considers that all the above-mentioned methodologies are suitable for official control for the bacterial identification *Lactiplantibacillus plantarum* DSM 34271 at strain level.

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.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control (i) DNA sequencing methods such as Whole Genome Sequencing (WGS) or Pulsed-Field Gel Electrophoresis (PFGE) (CEN/TS 17697) for the identification of *Lactiplantibacillus plantarum* DSM 34271; and (ii) the ring-trial validated spread plate (or pour plate) CEN method (EN 15787) for the enumeration of *Lactiplantibacillus plantarum* DSM 34271 in the *feed additive*.

Note: As the unambiguous determination of *Lactiplantibacillus plantarum* DSM 34271 added to silage is not achievable by analysis, the EURL cannot recommend the EN 15787 or any other method for official control to enumerate *Lactiplantibacillus plantarum* DSM 34271 in *silage*.

Recommended text for the register entry (analytical method)

- Identification: Pulsed-Field Gel Electrophoresis (PFGE) - CEN/TS 17697 or DNA sequencing methods
- Enumeration in the *feed additive*: Spread plate (or pour plate) method on MRS agar - EN 15787

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Lactiplantibacillus plantarum* DSM 34271 have been sent to the European Union Reference Laboratory for Feed Additives. The dossier has been made available to the EURL by EFSA.

6. REFERENCES

- [1] Forwarding of applications for authorisation of feed additives in accordance with Regulation (EC) No 1831/2003 – E-Submission Food Chain platform:
<https://webgate.ec.europa.eu/esfc/#/applications/38633>
<https://open.efsa.europa.eu/questions/EFSA-Q-2023-00250>
- [2] *Application, Annex 1
- [3] *Technical dossier, 2.1.3. Qualitative and quantitative composition, Detailed identification_confid.pdf
- [4] *Technical dossier, 2.2.1. Identification, Detailed identification_confid.pdf
- [5] *Technical dossier, 2.5.1. Proposed mode of use in animal nutrition, Conditions of use of the additive_non confid.pdf
- [6] EURL reports:
https://joint-research-centre.ec.europa.eu/publications/feed-2021-2210_en
https://joint-research-centre.ec.europa.eu/publications/fad-2020-0075007800790080_en
- [7] *Technical dossier, 2.6.1. Methods of analysis of the active substance, Methods of analysis and ref_confid.pdf
- [8] EN 15787:2021 – Animal feeding stuffs: Methods of sampling and analysis - Detection and enumeration of *Lactobacillus* spp. used as feed additive
- [9] ISO 7218:2007 – Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations
- [10] CEN/TS 17697:2023 - Animal feeding stuffs - Methods of sampling and analysis - PFGE typing of *Lactobacilli*, *Pediococci*, *Enterococci* and *Bacilli* in animal feeds

*Refers to Dossier no: FEED-2023-13173

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation is the European Union Reference Laboratory for Feed Additives, JRC, Geel, Belgium. This report is in accordance with the opinion of the consortium of National Reference Laboratories as referred to in Article 6(2) of Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761.

8. ACKNOWLEDGEMENTS

The following National Reference Laboratories contributed to this report:

- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, Pesca, Alimentació i Medi Natural. Generalitat de Catalunya, Cabrils (ES)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Laboratoire de Rennes (SCL L35), Service Commun des Laboratoires DGCCRF et DGDDI, Rennes (FR)