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**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**

Loigolactobacillus coryniformis DSM 34345
(FEED-2023-13176; CRL/230009)

**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**

Dossier related to: **FEED-2023-13176 - CRL/230009**

Name of Product : ***Loigolactobacillus coryniformis*
DSM 34345**

Active Agent (s): **Loigolactobacillus coryniformis
DSM 34345**

Rapporteur Laboratory: **European Union Reference Laboratory for
Feed Additives (EURL-FA)
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Date: **30/01/2024**

EXECUTIVE SUMMARY

In the current application an authorisation is sought under Article 4 for *Loigolactobacillus coryniformis* DSM 34345 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 *Loigolactobacillus coryniformis* DSM 34345 as *active substance* at a minimum concentration of 2×10^{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 *Loigolactobacillus coryniformis* DSM 34345 in the *feed additive* and in *silage* the Applicant proposed the ring-trial validated EN 15787 method. As the unambiguous determination of *Loigolactobacillus coryniformis* DSM 34345 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 *Lactoplantibacilli* reports, the EURL recommends for official control the ring-trial validated EN 15787 method for the enumeration of *Loigolactobacillus coryniformis* DSM 34345 in the *feed additive*.

For the identification of *Loigolactobacillus coryniformis* DSM 34345, 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 *Loigolactobacillus coryniformis* DSM 34345 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

Loigolactobacillus coryniformis DSM 34345, 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 *Loigolactobacillus coryniformis* DSM 34345 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 *Loigolactobacillus coryniformis* DSM 34345 as an *active substance* at a minimum concentration of 2×10^{11} Colony Forming Unit (CFU) / g [3].

The Applicant stated that *Loigolactobacillus coryniformis* DSM 34345 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 forage at a recommended dosage of 1×10^5 CFU / g fresh *silage* [5].

Note: The EURL, in the frame of former dossiers, has evaluated the analytical methods for the determination of other *Loigolactobacilli* strains (previously indicated as “lactobacilli”) [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 *Loigolactobacillus coryniformis* DSM 34345 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 *Loigolactobacillus coryniformis* DSM 34345 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 number of colony forming units (CFU) per g (or per ml) of feed is calculated according to the recommendations of the EN ISO 7218 standard [9] using an equation specified in the EN 15787 standard method [8]. 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].

In addition, depending on whether pour or spread plate methods are used, the lowest limit of quantification (LOQ) of 1x10⁵ CFU and 4x10⁵ CFU / kg *compound feed*, respectively can be derived [10] following the protocol of EN 15787 standard method and applying the provisions of EN ISO 7218 and EN 15787 methods, where the presence of minimum of 10 colonies/per plate are required for counting.

Based on the available information, the EURL recommends for official control the ring-trial validated EN 15787 method for the enumeration of *Loigolactobacillus coryniformis* DSM 34345 in the *feed additive*.

As the unambiguous determination of *Loigolactobacillus coryniformis* DSM 34345 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 *Loigolactobacillus coryniformis* DSM 34345 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 *Loigolactobacillus coryniformis* DSM 34345, 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 [6,11].

The EURL considers that all the above-mentioned methodologies are suitable for official control for the bacterial identification *Loigolactobacillus coryniformis* DSM 34345 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 *Loigolactobacillus coryniformis* DSM 34345; and (ii) the ring-trial validated spread plate (or pour plate) CEN method (EN 15787) for the enumeration of *Loigolactobacillus coryniformis* DSM 34345 in the *feed additive*.

Note: As the unambiguous determination of *Loigolactobacillus coryniformis* DSM 34345 added to silage is not achievable by analysis, the EURL cannot recommend the EN 15787 or any other method for official control to enumerate *Loigolactobacillus coryniformis* DSM 34345 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 *Loigolactobacillus coryniformis* DSM 34345 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/38636>
<https://open.efsa.europa.eu/questions/EFSA-Q-2023-00362>
- [2] *Application, Annex 1
- [3] *Technical dossier, 2.1.3. Qualitative and quantitative composition, Detailed identification_confid-1.pdf
- [4] *Technical dossier, 2.2.1. Identification, Detailed identification_confid-1.pdf
- [5] *Technical dossier, 2.5.1. Proposed mode of use in animal nutrition, Conditions of use of the additive_non confid-1.pdf
- [6] EURL reports (most recent):
https://joint-research-centre.ec.europa.eu/publications/feed-2023-13173_en
https://joint-research-centre.ec.europa.eu/publications/feed-2021-0246_en
https://joint-research-centre.ec.europa.eu/publications/feed-2021-2210_en
- [7] *Technical dossier, 2.6.1. Methods of analysis of the active substance, Methods of analysis and ref_confid-1.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] *Supplementary information – LOQ estimation by EURL_EN_15787
- [11] 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-2023-13176

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

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- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)