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JOINT RESEARCH CENTRE

Directorate F - Health, Consumers & Reference Materials (Geel)
European Union Reference Laboratory for Feed Additives

JRC.F.5/CvH/ZE/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**

***Lactobacillus plantarum* DSM26571**
(FAD-2019-0091; CRL/190052)



**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: **FAD-2019-0091 – CRL/190052**

Name of Product: ***Lactobacillus plantarum* DSM26571**

Active Agent (s): ***Lactobacillus plantarum* DSM26571**

Rapporteur Laboratory: **Centre Wallon de Recherches
Agronomiques (CRA-W), Gembloux,
Belgium**

Report prepared by: **Véronique Ninane**

Report checked by: **Zigmas Ezerskis**
Date: **15/10/2020**

Report approved by: **Christoph von Holst**
Date: **15/10/2020**

EXECUTIVE SUMMARY

In the current application an authorisation is sought under Article 4(1) for *Lactobacillus plantarum* DSM26571 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 as *active substance* viable cells of the non-genetically modified strain *Lactobacillus plantarum* DSM26571. The *feed additive* is to be marketed as a preparation containing a minimum content of *active substance* of 1×10^{11} Colony Forming Unit (CFU)/g and to be added to *silage* as such or mixed with other additives, at a minimum dose of 1×10^5 CFU/g fresh *silage*.

For the identification of *Lactobacillus plantarum* DSM26571 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 *Lactobacillus plantarum* DSM26571 in the *feed additive* the EURL recommends for official control the ring-trial validated spread plate method EN 15787.

Since the enumeration of *Lactobacillus plantarum* DSM26571 initially added to *silage* is not achievable by analysis, the EURL cannot recommend the method EN 15787 or any other method for official control to enumerate *Lactobacillus plantarum* DSM26571 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.

KEYWORDS

Lactobacillus plantarum DSM26571, 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 *Lactobacillus plantarum* DSM26571 under the category / functional group 1(k) 'technological additives' / 'silage additives', according to Annex I of Regulation (EC) No 1831/2003 [1]. 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 as *active substance* viable cells of the non-genetically modified strain *Lactobacillus plantarum* DSM26571 [3].

Lactobacillus plantarum is an old synonymous name to *Lactiplantibacillus plantarum* - the new name published for the species [4].

The strain is deposited at the Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany) under the deposit number DSM26571 [5].

The *feed additive* is to be marketed as a preparation containing a minimum content of *active substance* of 1×10^{11} Colony Forming Unit (CFU)/g [6].

The *feed additive* is intended to be added to *silage* as such or mixed with other additives at a minimum dose of 1×10^5 CFU/g fresh *silage* [7].

Note: According to the Applicant mixed additives may contain other strains of lactic acid bacteria than *Lactobacillus plantarum* DSM26571 [8].

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 *Lactobacillus plantarum* DSM26571 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 and silage (section 2.6.1 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

For the enumeration of *Lactobacillus plantarum* DSM26571 in the *feed additive*, the Applicant applied an in-house plate count method, which was neither validated nor further verified [9]. The method is based on the ring-trial validated spread plate method EN 15787 developed by CEN for the analysis of *Lactobacillus* spp. [10].

According to the EN 15787 method the sample is suspended in phosphate buffered saline and further diluted in peptone salt solution; the appropriate dilutions are then spread on MRS (de Man, Rogosa, Sharp) agar plates. The agar plates are incubated anaerobically at 37 °C for 48 to 72 hours.

The performance characteristics reported from the validation study of the method EN 15787 after logarithmic transformation of the CFU values [10] are:

- a standard deviation for *repeatability* (S_r) of 0.24 \log_{10} CFU/g, and
- a standard deviation for *reproducibility* (S_R) ranging from 0.29 to 0.38 \log_{10} CFU/g.

In addition, the EURL calculated a limit of quantification (LOQ) of 3×10^3 CFU/g following the recommendations of ISO 7218 [11].

Based on the performance characteristics presented, the EURL recommends for official control the ring-trial validated EN 15787 method for the enumeration of *Lactobacillus plantarum* DSM26571 in the *feed additive*.

The Applicant did not provide any experimental method or data for the quantification of *Lactobacillus plantarum* DSM26571 in *silage*. Furthermore, the unambiguous determination of *Lactobacillus plantarum* DSM26571 initially added to *silage* is not achievable by analysis. Therefore, the EURL cannot recommend the method EN 15787 or any other method for official control to enumerate the microorganism of concern in *silage*.

Note: Mixed with other additives consisting of lactic acid bacteria, the accurate enumeration of *Lactobacillus plantarum* DSM26571 in the corresponding products may be not achievable.

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 identification of *Lactobacillus plantarum* DSM26571 the EURL recommends for official control Pulsed-Field Gel Electrophoresis (PFGE), a generally recognised methodology for the genetic identification of bacterial strains [12]. This methodology for microbial identification of authorised feed additives at strain level is currently being evaluated by the CEN Technical Committee 327 to become a European Standard.

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 the official control Pulsed-Field Gel Electrophoresis (PFGE) for the identification of *Lactobacillus plantarum* DSM26571 and the ring-trial validated spread plate method EN 15787 for the enumeration of *Lactobacillus plantarum* DSM26571 in the *feed additive*.

Recommended text for the register entry (analytical method)

- Identification: Pulsed Field Gel Electrophoresis (PFGE)
- Enumeration in the *feed additive*: Spread 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 *Lactobacillus plantarum* DSM26571 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] *Application, Reference SANTE E5: F.A. 1831/0027-2020 & Annex 1 – submission number 1575275846243-2478
- [2] *Application, Proposal for Register Entry, Annex A
- [3] *Technical dossier, Section II: 2.2 Characterisation of the Active Substance
- [4] Zheng J., Wittouck S., Salvetti E., Franz C.M.A.P., Harris H.M.B., Mattarelli P., O’Toole P.W., Pot B., Vandamme P., Walter J. *et al.* A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901 and union of *Lactobacillaceae* and *Leuconostocaceae*. *Int. J. Syst. Evol. Microbiol.*, 2020

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- [5] *Technical dossier, Section II: Annex II.2.1.2b
 - [6] *Technical dossier, Section II: 2.1.3 Qualitative and quantitative composition
 - [7] *Technical dossier, Section II: 2.5 Conditions of use of the additive
 - [8] *Technical dossier, Section II: 2.3 Manufacturing process, including any specific processing procedures
 - [9] *Technical dossier, Section II – Annex_II.6.1_SOP-03174
 - [10] EN 15787 - Animal feeding stuffs - Isolation and enumeration of *Lactobacillus* spp.
 - [11] EN ISO 7218:2007 - Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations
 - [12] European Community Project SMT4-CT98-2235."Methods for the Official Control of Probiotics Used as Feed Additives", Report 20873/1 EN (2002) ISBN 92-894-6250-7 (Vol. I) and Report 20873/3 EN (2002) ISBN 92-894-6252-3 (Vol. III)

*Refers to Dossier no: FAD-2019-0091

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was Centre Wallon de Recherches Agronomiques (CRA-W), Gembloux, 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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- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, Pesca, Alimentació i Medi Natural. Generalitat de Catalunya, Cabrils (ES)
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