



EUROPEAN COMMISSION

DIRECTORATE GENERAL

JOINT RESEARCH CENTRE

Directorate D: Institute for Reference Materials and Measurements

European Union Reference Laboratory for Feed Additives

 Ref. Ares(2016)1844602 - 19/04/2016

JRC.DG.D. 5/CvH/SB/mds/Ares (2016)

**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 rhamnosus DSM 29226
(FAD-2015-0033; CRL/150020)



**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-2015-0033 – CRL/150020**

Name of Product: ***Lactobacillus rhamnosus DSM 29226***

Active Agent (s): **Lactobacillus rhamnosus DSM 29226**

Rapporteur Laboratory: **Centre wallon de Recherches
agronomiques (CRA-W), Gembloux,
Belgium**

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Date: **19/04/2016**

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Date: **19/04/2016**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4(1) for *Lactobacillus rhamnosus* DSM 29226 under the category / functional group 1(k) 'technological additives' / 'silage additives', according to Annex I of Regulation (EC) No 1831/2003. 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 rhamnosus* DSM 29226. The *feed additive* is to be marketed as a powder or granules containing a minimum *Lactobacillus rhamnosus* DSM 29226 concentration of 1×10^{10} Colony Forming Unit (CFU)/g. The *feed additive* is intended to be added to *silage* at a minimum dose of 5×10^4 CFU/g fresh *silage*.

For the identification of *Lactobacillus rhamnosus* DSM 29226, the EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised standard methodology for genetic identification. This standard methodology for microbial identification is currently being evaluated by the CEN Technical Committee 327 to become European Standard.

For the enumeration of *Lactobacillus rhamnosus* DSM 29226 in the *feed additive per se*, the Applicant submitted the ring-trial validated spread plate method EN 15787. Based on the performance characteristics available, the EURL recommends this method for official control.

Since the enumeration of added *Lactobacillus rhamnosus* DSM 29226 in silage is not achievable by analysis, the EURL cannot recommend any method for official control.

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) is not considered necessary.

KEYWORDS

Lactobacillus rhamnosus DSM 29226, technological additives, silage additives, all species.

1. BACKGROUND

In the current application authorisation is sought under Article 4(1) for *Lactobacillus rhamnosus* DSM 29226 under the category / functional group 1(k) 'technological additives' / 'silage additives', according to Annex I of Regulation (EC) No 1831/2003 [1]. 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 rhamnosus* DSM 29226. The strain is deposited at DSMZ GmbH (DE) [3].

The *feed additive* is to be marketed as a powder or granules containing a minimum *Lactobacillus rhamnosus* DSM 29226 concentration of 1×10^{10} Colony Forming Unit (CFU)/g [4].

The *feed additive* is intended to be sprayed on *silage*, or spread via a dry carrier, at a minimum dose of 5×10^4 CFU/g fresh *silage* [2,3,5].

Note: The EURL previously evaluated the analytical methods for the determination of *Lactobacillus rhamnosus* in the frame of several dossiers (e.g. FAD-2006-0014; FAD-2010-0305; FAD-2010-0405; FAD-2014-0022) [6].

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009, 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 rhamnosus* DSM 29226 and their suitability to be used for official controls in the frame of the authorisation were evaluated.

3. EVALUATION

Identification /Characterisation of the feed additive

For the identification of *Lactobacillus rhamnosus* DSM 29226, the Applicant applied 16S rRNA gene sequence analysis and multi locus sequence typing [3].

The EURL recommends instead for official control the PFGE, a generally recognised standard methodology for genetic identification [7]. This standard methodology for microbial identification is currently being evaluated by the CEN Technical Committee 327 to become European Standard.

Qualitative and quantitative composition of impurities in the additive

The Applicant analysed the *feed additive* for microbial contaminants (e.g. yeast and mould *Escherichia coli*, presumptive coliforms and *Salmonella*) using the methods mentioned in the technical dossier [3]. As for the determination of other undesirable substances in the *feed additive* (e.g. arsenic, cadmium, lead, mercury, mycotoxins), analytical methods for official control are available from the respective European Union Reference Laboratories [8].

Description of the analytical methods for the determination of the active substances in feed additive and silage

For the enumeration of *Lactobacillus rhamnosus* DSM 29226 in the *feed additive* and *silage*, the Applicant submitted the ring-trial validated spread plate method EN 15787 developed by CEN [2,3].

The sample is suspended and diluted in a buffer 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 following performance characteristics were reported after logarithmic transformation (CFU) [9]:

- a standard deviation for repeatability (s_r) of 0.24 log₁₀ CFU/g,
- a standard deviation for reproducibility (s_R) ranging from 0.29 to 0.38 log₁₀ CFU/g, and
- a limit of quantification (LOQ) of 3x10³ CFU/g [10].

Based on the performance characteristics presented, the EURL recommends for official control the ring-trial validated EN 15787 method for the enumeration of *Lactobacillus rhamnosus* DSM 29226 in *feed additive per se*.

Since the unambiguous determination of *Lactobacillus rhamnosus* DSM 29226 added to silage is not achievable by analysis, the EURL cannot recommend the EN 15787 or any other method for official control to quantify the micro-organism of concern 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) is not considered necessary.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE) for the identification of *Lactobacillus rhamnosus* DSM 29226 and the ring-trial validated spread plate method EN 15787 for the enumeration of this strain in the *feed additive*.

Since the unambiguous determination of *Lactobacillus rhamnosus* DSM 29226 added to silage is not achievable by analysis, the EURL cannot recommend the EN 15787 or any other method for official control to quantify the micro-organism of concern in *silage*.

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 rhamnosus* DSM 29226 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 G1: F.A. 1831/0024-2015
- [2] *Application, Proposal for Register Entry, Annex A
- [3] *Technical dossier, Section II, Identity, characterisation and conditions of use of the additive
- [4] *Suppl. Information dated 06/04/2016: 201604061200.pdf
- [5] *Technical dossier, Section I, Public summary
- [6] EURL Evaluation reports :
<https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2006-0014.pdf>
<https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2010-0305.pdf>
<https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2010-0405.pdf>
<https://ec.europa.eu/jrc/sites/default/files/finrep-fad-2014-0022-procanius.pdf>
- [7] 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)
- [8] Commission Regulation (EC) No 776/2006 amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards to Community Reference Laboratories

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- [9] EN 15787:2009 - Animal feeding stuffs - Isolation and enumeration of Lactobacillus spp.
- [10] EN ISO 7218:2007 - Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations

*Refers to Dossier no: FAD-2015-0033

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 (EC) No 885/2009.

8. ACKNOWLEDGEMENTS

The following National Reference Laboratories contributed to this report:

- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- 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)
- Laboratoire de Rennes (SCL L35), Service Commun des Laboratoires DGCCRF et DGDDI, Rennes (FR)
- Univerza v Ljubljani. Veterinarska fakulteta. Nacionalni veterinarski inštitut. Enota za patologijo prehrane in higieno okolja, Ljubljana (SI)