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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**

Lactobacillus plantarum DSM 29025
(FAD-2015-0035; CRL/150021)

**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-2015-0035 - CRL/150021**

Name of Product : ***Lactobacillus plantarum DSM 29025***

Active Agent (s): **Lactobacillus plantarum DSM 29025**

Rapporteur Laboratory: **Laboratoire de Rennes (SCL L35), Service
Commun des Laboratoires DGCCRF et
DGDDI, Rennes, France**

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Date: **21/04/2016**

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Date: **21/04/2016**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4(1) for *Lactobacillus plantarum* DSM 29025 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 *active substance* in the *feed additive* consists in viable cells of the non-genetically modified strain *Lactobacillus plantarum* DSM 29025. The *feed additive* is to be marketed as a powder containing a minimum *Lactobacillus plantarum* DSM 29025 concentration of 8×10^{10} Colony Forming Unit (CFU)/g. The *feed additive* is intended to be added to *silage* at a minimum dose of 5×10^7 CFU/kg fresh *silage*.

For the identification of *Lactobacillus plantarum* DSM 29025, the EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE), a recognised standard methodology for genetic identification. This methodology for microbial identification is currently being evaluated by the CEN Technical Committee 327 to become a European Standard.

For the enumeration of *Lactobacillus plantarum* DSM 29025, the Applicant submitted the ring-trial validated spread plate method EN 15787 which was already evaluated by EURL in the frame of previous *Lactobacillus plantarum* dossiers. Based on the performance characteristics available, the EURL recommends for official control this ring-trial validated EN 15787 method for the enumeration of *Lactobacillus plantarum* DSM 29025 in the *feed additive per se*.

The Applicant did not provide any data or experimental method for the determination of *Lactobacillus plantarum* DSM 29025 in *silage*, since the unambiguous determination of the content of *Lactobacillus plantarum* DSM 29025 added to *silage* is not achievable by analysis. Therefore, the EURL cannot evaluate nor recommend any method for official control to determine *Lactobacillus plantarum* DSM 29024 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.

KEYWORDS

Lactobacillus plantarum DSM 29025, technological additives, silage additives, all species.

1. BACKGROUND

In the current application authorisation is sought under Article 4(1) for *Lactobacillus plantarum* DSM 29025 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 *Lactobacillus plantarum* DSM 29025. The strain is deposited at DSMZ German Collection of Microorganisms and Cell Cultures under the number *Lactobacillus plantarum* DSM 29025 [2,3].

The *feed additive* is to be marketed as a powder containing a minimum *Lactobacillus plantarum* DSM 29025 concentration of 8×10^{10} Colony Forming Unit (CFU)/g [4,5].

The *feed additive* is intended to be added dry or via a water suspension to *silage* at a minimum dose of 5×10^7 CFU/kg fresh *silage* [2,6].

Note: The EURL previously evaluated the analytical methods for the determination of *Lactobacillus plantarum* in the frame of several dossiers e.g. FAD 2011-0004, FAD-2010-0109, FAD-2010-0048 and FAD-2015-0013 [7].

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* DSM 29025 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 plantarum* DSM 29025, the Applicant used a combination of methods: 16s ribosomal testing and multi locus sequence typing (MLST) [3]. The EURL recommends instead for official control the Pulsed Field Gel Electrophoresis (PFGE), a generally recognised standard methodology for genetic identification [8]. This

standard methodology for microbial identification is currently being evaluated by the CEN Technical Committee 327 to become a European Standard.

Qualitative and quantitative composition of impurities in the feed additive

The Applicant analysed the *feed additive* for microbial contaminants (e.g. *Escherichia coli*, Presumptive Coliforms, *Salmonella* spp. and Yeast and Moulds) using the methods described in the technical dossier [9,10]. For the determination of the undesirable substances in the *feed additive* (e.g. arsenic, cadmium, lead, mercury, aflatoxin B1), analytical methods are available from the respective European Union Reference Laboratories [11].

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

For enumeration of *Lactobacillus plantarum* DSM 29025 in *feed additive* and *silage*, the Applicant submitted the ring-trial validated spread plate method (EN 15787), developed by CEN for enumeration of *Lactobacillus* spp [10,12].

The sample is suspended and diluted in PBS (phosphate buffered saline), the appropriated dilutions are then spread on MRS (de Man, Rogosa and Sharp) agar plates. The agar plates are incubated anaerobically at 37°C for 48 to 72 hours before enumeration. The performance characteristics of the EN 15787 method reported after logarithmic transformation of measured values (CFU) are [12]:

- 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;
- a limit of quantification (LOQ) of 3x10³ CFU/g of *feed additive* [13], well below the minimum dose proposed by the Applicant [2].

Based on the performances characteristics presented, the EURL recommends for official control the CEN method (EN15787) for the determination of *Lactobacillus plantarum* DSM 29025 in the *feed additive per se*.

Since the unambiguous determination of *Lactobacillus plantarum* DSM 29025 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 the CEN method (EN15787) for the enumeration of the active agent *Lactobacillus plantarum* DSM 29025 in the *feed additive* and Pulsed Field Gel Electrophoresis (PFGE) for its identification.

The Applicant did not provide any experimental method or data for determination of *Lactobacillus plantarum* DSM 29025 in silage. Furthermore, the unambiguous determination of the content of *Lactobacillus plantarum* DSM 29025 added to *silage* is not achievable by analysis. Therefore the EURL cannot evaluate nor recommend any method for official control to determine *Lactobacillus plantarum* DSM 29025 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 (EN15787)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Lactobacillus plantarum* DSM 29025 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 SANCO/G1: Forw. Appl. 1831/0025-2015
- [2] *Application, Proposal for Register Entry – Annex A
- [3] *Technical dossier, Section II: 2.2 Characterisation of the Active Ingredient
- [4] *Application Form, Annex 1 of Regulation 429/2008
- [5] *Suppl. Information dated 06/04/2016: 201604061200.pdf
- [6] *Technical dossier, Section II: 2.5 Conditions of use of the additive
- [7] EURL Evaluation Reports:
<https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2011-0004.pdf>
<https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2010-0109.pdf>
<https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2010-0048.pdf>
<https://ec.europa.eu/jrc/sites/default/files/finrep-FAD-2015-0013-lactobacillus%20plantarum.pdf>
- [8] 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)
- [9] *Technical dossier, Section II: 2.1.4 Purity

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- [10] *Technical dossier, Section II: 2.6 Methods of Analysis
- [11] 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
- [12] EN 15787:2009 – Animal feeding stuffs – Isolation and enumeration of *Lactobacillus*
- [13] ISO 7218:1996 – Microbiology of food and animal feeding stuffs – General rules for microbiological examinations and Annexe A1 October 2013
- *Refers to Dossier no: FAD-2015-0035

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was "Laboratoire de Rennes (SCL L35), Service Commun des Laboratoires DGCCRF et DGDDI, Rennes, France". 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:

- Centre wallon de Recherches agronomiques (CRA-W), Gembloux (BE)
- 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)
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- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, PESCA, Alimentació i Medi Natural. Generalitat de Catalunya, Cabrils (ES)
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