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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 29024**  
*(FAD-2015-0034; CRL/150019)*



**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-2015-0034 - CRL/150019**

Name of Product : ***Lactobacillus plantarum DSM 29024***

Active Agent (s): **Lactobacillus plantarum DSM 29024**

Rapporteur Laboratory: **Centro di referenza nazionale per la  
sorveglianza ed il controllo degli alimenti  
per gli animali (CReAA), Torino, Italy**

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Date: **21/04/2016**

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Date: **21/04/2016**

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## EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4(1) for *Lactobacillus plantarum* DSM 29024 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 29024. The *feed additive* is to be marketed as a powder containing a minimum *Lactobacillus plantarum* DSM 29024 concentration of  $8 \times 10^{10}$  Colony Forming Unit (CFU)/g. The *feed additive* is intended to be added to *silage* at a minimum dose of  $5 \times 10^7$  CFU/kg fresh *silage*.

For the identification of *Lactobacillus plantarum* DSM 29024, 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 29024, 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 29024 in the *feed additive per se*.

The Applicant did not provide any data or experimental method for the determination of *Lactobacillus plantarum* DSM 29024 in *silage*, since the unambiguous determination of the content of *Lactobacillus plantarum* DSM 29024 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 29024, technological additives, silage additives, all species.

## 1. BACKGROUND

In the current application authorisation is sought under Article 4(1) for *Lactobacillus plantarum* DSM 29024 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 plantarum* DSM 29024. The strain is deposited at DSMZ German Collection of Microorganisms and Cell Cultures under the number *Lactobacillus plantarum* DSM 29024 [2,3].

The *feed additive* is to be marketed as a powder containing a minimum *Lactobacillus plantarum* DSM 29024 concentration of  $8 \times 10^{10}$  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  $5 \times 10^7$  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 29024 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 29024 the Applicant used sequencing analysis of 16S rRNA gene 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 European Standard.

#### *Qualitative and quantitative composition of impurities in the additive*

The Applicant analysed the *feed additive* for microbial contaminants (e.g. Escherichia coli, presumptive Coliforms and Salmonella, 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 and dioxins), 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 29024 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 a buffer solution; the appropriate dilutions are then spread on MRS (de Man, Rogosa and Sharp) agar plates. The agar plates are incubated at 37 °C for 48 to 72 hours and must be placed in anaerobiosis for enumeration of lactobacilli. The following performance characteristics were reported after logarithmic transformation (CFU) [12]:

- a standard deviation for repeatability ( $s_r$ ) of 0.24 log<sub>10</sub> CFU/g;
- a standard deviation for reproducibility ( $s_R$ ) ranging from 0.29 to 0.38 log<sub>10</sub> CFU/g;
- a limit of quantification (LOQ) of 3x10<sup>3</sup> CFU/g [13], well below the minimum dose proposed by the Applicant [2].

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* DSM 29024 in *feed additive per se*.

Since the unambiguous determination of *Lactobacillus plantarum* DSM 29024 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 plantarum* DSM 29024 and the ring-trial validated spread plate method EN 15787 for the enumeration of the strain in the feed additive.

The Applicant did not provide any experimental method or data for the determination of *Lactobacillus plantarum* DSM 29024 in *silage*, since the unambiguous determination of the content of *Lactobacillus plantarum* DSM 29024 added to *silage* is not achievable experimentally. Therefore, the EURL cannot evaluate nor recommend any method for official control to determine *Lactobacillus plantarum* DSM 29024 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 plantarum* DSM 29024 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. 1831/0026-2015
- [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: II.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

- [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] EN ISO 7218:2007 - Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations

\*Refers to Dossier no: FAD-2015-0034

## **7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES**

The Rapporteur Laboratory for this evaluation was "Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino, Italy". 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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