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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 buchneri DSM 29026

(FAD-2018-0093; CRL/180063)

European Union Reference Laboratory
Feed Additives

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-2018-0093 - CRL/180063**
Name of Product:	***Lactobacillus buchneri DSM 29026***
Active Agent (s):	**Lactobacillus buchneri DSM 29026**
Rapporteur Laboratory:	**Centre wallon de Recherches agronomiques (CRA-W), Gembloux, Belgium**
Report prepared by:	**Véronique Ninane**
Report checked by: Date:	**M. J. González de la Huebra & Z. Ezerskis** **17/07/2019**
Report approved by: Date:	**Christoph von Holst** **17/07/2019**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4(1) for a preparation of *Lactobacillus buchneri* DSM 29026 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* in *silage* for all animal species.

According to the Applicant, the *feed additive* contains as *active substance* viable spores of the non-genetically modified strain *Lactobacillus buchneri* DSM 29026. The *feed additive* is to be marketed as a powder preparation containing a minimum *Lactobacillus buchneri* DSM 29026 content of $2x10^{10}$ Colony Forming Unit (CFU)/g. The *feed additive* is intended to be added to *silage* at a minimum dose of $5x10^{5}$ CFU/g of fresh *silage* if used alone, or at a minimum dose of $5x10^{4}$ CFU/g of fresh *silage* if combined with other microorganisms.

For the identification of *Lactobacillus buchneri* DSM 29026, 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 buchneri* DSM 29026 in the *feed additive*, 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.

The Applicant did not provide any experimental method or data for the quantification of *Lactobacillus buchneri* DSM 29026 in *silage*. Since the unambiguous enumeration of *Lactobacillus buchneri* DSM 29026 initially added to *silage* is not achievable by analysis. Therefore, the EURL cannot evaluate nor recommend any method for official control to quantify the active substance 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 buchneri DSM 29026, technological additives, silage additives, all animal species

1. BACKGROUND

In the current application authorisation is sought under Article 4(1) (authorisation of a new feed additive) for a preparation of *Lactobacillus buchneri* DSM 29026 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* in *silage* for all animal species [2].

According to the Applicant, the *feed additive* contains as *active substance* viable spores of the non-genetically modified strain *Lactobacillus buchneri* DSM 29026 [3]. The strain is deposited at the Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany) under the deposit number DSM 29026 [4].

The *feed additive* is to be marketed as a powder preparation containing a minimum *Lactobacillus buchneri* DSM 29026 content of $2x10^{10}$ Colony Forming Unit (CFU)/g [5].

The *feed additive* is intended to be added alone to *silage* at a minimum dose of $5x10^{5}$ CFU/g of fresh *silage*, or with other microorganisms at a minimum dose of $5x10^{4}$ CFU/g of fresh *silage* [6].

Note: The EURL previously evaluated the analytical methods for the determination of *Lactobacillus* spp. in the frame of several dossiers [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 buchneri* DSM 29026 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, feedingstuffs and when appropriate water (section 2.6.1 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

For the enumeration of *Lactobacillus buchneri* DSM 29026 in the *feed additive* the Applicant submitted the ring-trial validated spread plate CEN method EN 15787 [8] which was already evaluated by the EURL in the frame of previous *Lactobacillus* spp. dossiers [7].

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, expressed in terms of precision, were reported after logarithmic transformation of the CFU values [8]:

- 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 $3x10^4$ CFU/g following the recommendations of the ISO 7218 standard [9].

Based on the performance characteristics presented, the EURL recommends for official control the ring-trial validated 15787 method for the enumeration of *Lactobacillus buchneri* DSM 29026 in the *feed additive*.

The Applicant did not provide any experimental method or data for the quantification of *Lactobacillus buchneri* DSM 29026 in *silage*. Furthermore, the unambiguous enumeration of *Lactobacillus buchneri* DSM 29026 initially added to *silage* is not achievable by analysis. Therefore, the EURL cannot evaluate nor recommend any method for official control to quantify *Lactobacillus buchneri* DSM 29026 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 identification of *Lactobacillus buchneri* DSM 29026 at strain level, the Applicant applied multi-locus sequence typing (MLST) [10]. The EURL recommends instead for official control Pulsed-Field Gel Electrophoresis (PFGE), a generally recognised methodology for the genetic identification of bacterial strains [11]. A PFGE method for microbial identification of authorised feed additives at strain level is currently being evaluated by the CEN Technical Committee 327 to become an 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 official control Pulsed Field Gel Electrophoresis (PFGE) for the identification of *Lactobacillus buchneri* DSM 29026 and the ring-trial validated spread plate method EN 15787 for the enumeration of this strain in the *feed additive.*

The Applicant did not provide any experimental method or data for the quantification of *Lactobacillus buchneri* DSM 29026 in *silage*. Furthermore, the unambiguous enumeration of *Lactobacillus buchneri* DSM 29026 initially added to *silage* is not achievable by analysis. Therefore, the EURL cannot evaluate nor recommend any method for official control to quantify *Lactobacillus buchneri* DSM 29026 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 buchneri* DSM 29026 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. 1831/0013-2019

[2] *Application, Proposal for Register Entry, Annex A

[3] *Technical dossier, Section II: 2.2 Characterisation of the Active Substance

[4] *Technical dossier, Section II: Annex II.8.

[5] *Application, Description and conditions of use of the additive, Annex A

[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-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

https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2016-0050-lacto_hilgardii.pdf
https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2016-0060-lactobacillus_buchneri.pdf
https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2017-0002-lacto_reuteri.pdf
https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2018-0015-lactobacillushilbuc.pdf

[8] EN 15787:2009 - Animal feeding stuffs - Isolation and enumeration of Lactobacillus spp.

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

[10] *Technical dossier, Section II: 2.2 Characterisation of the active ingredient

[11] 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-2018-0093

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation is the 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)
- Instytut Zootechniki - Państwowy Instytut Badawczy, Krajowe Laboratorium Pasz, Lublin (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)