



EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

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

JRC F.5/CvH/SB/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 JI:1 (DSM 115 20)
(FAD-2017-0004; CRL/100366)



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in connection with the Application for Authorisation of a
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Dossier related to: **FAD-2017-0004 - CRL/100366**

Name of Product: ***Lactobacillus plantarum JI:1 (DSM 115 20)***

Active Agent (s): **Lactobacillus plantarum JI:1 (DSM 115 20)**

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

Report prepared by: **Stefania Squadrone**

Report checked by: **Stefano Bellorini**
Date: **29/01/2020**

Report approved by: **Christoph von Holst**
Date: **29/01/2020**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4(1) (authorisation of a new feed additive) for a preparation of *Lactobacillus plantarum* JI:1 (DSM 115 20) under the category / functional group 4(b) 'zootechnical additives' / 'gut flora stabilisers', according to Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for the use of the *feed additive* for horses.

According to the Applicant, the *feed additive* contains as *active substance* viable cells of the non-genetically modified strain *Lactobacillus plantarum* JI:1 (DSM 115 20). The *feed additive* is to be marketed as liquid preparation (*ProEquo*[®] liquid) or as a 6 g dry feed-stick (*ProEquo*[®] Sticks). The dry form of the *feed additive* has a minimum *Lactobacillus plantarum* JI:1 (DSM 115 20) content of 13x10⁹ Colony Forming Unit (CFU)/g. The *feed additive* is intended to be added poured on top of other feed

For the enumeration of *Lactobacillus plantarum* JI:1 (DSM 115 20) in the *feed additive* the Applicant submitted the NMKL method No. 140 (1991) published by the Nordic Committee on Food Analysis. Nevertheless, the Applicant did not provide any experimental data demonstrating the fitness for purpose of the proposed method for the enumeration of *Lactobacillus plantarum* JI:1 (DSM 115 20) in the *feed additive*.

The EURL requested the Applicant to apply and verify the ring-trial validated spread plate CEN method EN 15787 for the enumeration of *lactobacilli* in the two commercial products (*ProEquo*[®] liquid and *ProEquo*[®] Sticks). However the Applicant did not follow the EURL request and confirmed the submission of the incomplete information as provided in the original dossier. Therefore the EURL cannot conclude on the suitability of the proposed methods of analysis (i.e. EN 15787 and/or NMKL method No. 140/1991) for the official control of *Lactobacillus plantarum* JI:1 (DSM 115 20) in the *feed additive*.

For the enumeration of *Lactobacillus plantarum* JI:1 (DSM 115 20) in the *feedingstuff* the Applicant submitted a procedure based on the abovementioned NMKL method No. 140 (1991). However the Applicant did not provide any supporting experimental data. Therefore, the EURL cannot evaluate and is unable to recommend any method for official control to quantify *Lactobacillus plantarum* JI:1 (DSM 115 20) in *feed*.

For the identification of *Lactobacillus plantarum* JI:1 (DSM 115 20), the EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised methodology for the genetic identification of bacterial strains.

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 JI:1 (DSM 115 20), zootechnical additives, gut flora stabilisers, horses

1. BACKGROUND

In the current application authorisation is sought under Article 4(1) (authorisation of a new feed additive) for a preparation of *Lactobacillus plantarum* JI:1 (DSM 115 20) under the category / functional group 4(b) 'zootechnical additives' / 'gut flora stabilisers', according to Annex I of Regulation (EC) No 1831/2003 [1-2]. Authorisation is sought for the use of the *feed additive* for horses [1,3].

According to the Applicant, the *feed additive* contains as *active substance* viable cells of the non-genetically modified strain *Lactobacillus plantarum* JI:1 (DSM 115 20) [4]. The strain is deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany) under the deposit number DSM 115 20 [5].

The *feed additive* is to be marketed as liquid preparation (ProEquo[®] liquid) or as a 6 g dry feed-stick (ProEquo[®] Sticks) [6]. The dry form of the feed additive has a minimum *Lactobacillus plantarum* JI:1 (DSM 115 20) content of 13×10^9 Colony Forming Unit (CFU)/g [4]. The *feed additive* is intended to be added poured on top of other feed [7].

Note: The EURL previously evaluated the analytical methods for the determination of *Lactobacillus* spp. in the frame of several dossiers [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* JI:1 (DSM 115 20) 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 plantarum* JI:1 (DSM 115 20) in the *feed additive* the Applicant submitted the NMKL method No. 140 (1991) "Lactic acid bacteria. Determination in meat and meat products" published by the Nordic Committee on Food Analysis [9,10]. Nevertheless, the Applicant did not provide any experimental data demonstrating the fitness for purpose of the proposed method for the enumeration of *Lactobacillus plantarum* JI:1 (DSM 115 20) in the *feed additive* [9,11].

In the frame of previous *Lactobacillus* spp. dossiers the EURL positively evaluated the ring-trial validated spread plate CEN method EN 15787 [8,12]. The Applicant was therefore invited by the EURL to apply the CEN method for the enumeration of *lactobacilli* in the two commercial products (*ProEquo*[®] *liquid* and *ProEquo*[®] *Sticks*) by conducting appropriate verification experiments [13]. However the Applicant did not follow the EURL request and answered confirming the submission of the incomplete information as provided in the original dossier [4].

On the basis of the data submitted by the Applicant, the EURL cannot conclude on the suitability of the methods of analysis (i.e. EN 15787 and/or NMKL method No. 140/1991) for the official control of *Lactobacillus plantarum* JI:1 (DSM 115 20) in the *feed additive*.

For the enumeration of *Lactobacillus plantarum* JI:1 (DSM 115 20) in the *feedingstuff* the Applicant submitted a procedure based on the abovementioned NMKL method No. 140 (1991) [10,14]. However the Applicant did not provide any supporting experimental data [15]. Therefore, the EURL cannot evaluate and is unable to recommend any method for official control to quantify *Lactobacillus plantarum* JI:1 (DSM 115 20) in *feed*.

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* JI:1 the Applicant proposed a method based on the evaluation of the fermentation profile on API 50CH of an isolated colony [16]. The EURL recommends instead for official control Pulsed-Field Gel Electrophoresis (PFGE), a generally recognised methodology for the genetic identification of bacterial strains [17]. 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 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 official control Pulsed Field Gel Electrophoresis (PFGE) for the identification of *Lactobacillus plantarum* JI:1 (DSM 115 20).

On the basis of the data submitted by the Applicant, the EURL cannot conclude on the suitability of the method of analysis for the official control of *Lactobacillus plantarum* JI:1 (DSM 115 20) in the *feed additive*.

The unambiguous enumeration of *Lactobacillus plantarum* JI:1 (DSM 115 20) initially added to *feed* is not achievable by analysis. Therefore, the EURL cannot evaluate and is unable to recommend any method for official control to quantify *Lactobacillus plantarum* JI:1 (DSM 115 20) in *feed*.

Recommended text for the register entry (analytical method)

- Identification: Pulsed Field Gel Electrophoresis (PFGE)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Lactobacillus plantarum* JI:1 (DSM 115 20) 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/D/2 : FORW.APPL. 1831(00204) (10427)-2010
- [2] *Application, Partial withdrawal, Reference FAD-2017-0004 / SANTE-0204-02010
- [3] *Application, Description and condition of use of the additive
- [4] *Supplementary information, EFSA-Q-2017-00023_FAD-2017-004_Suppl_info.pdf
- [5] *Technical dossier, Section II: 2.2. Characterization of the active substance
- [6] *Technical dossier, Section II : 2.3. Manufacturing Process
- [7] *Technical dossier, Section II: 2.5.1. Proposed mode of use in animal nutrition
- [8] EURL Evaluation Reports:
<https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2006-0014.pdf>

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- [9] *Technical dossier, Section II: 2.6.1. Methods of analysis for the active substance
- [10] Nordic Committee on Food Analysis: <https://www.nmkl.org/index.php/en/>
- [11] *Technical dossier, Section II: Annex II-4 Method of analysis.pdf
- [12] EN 15787:2009 - Animal feeding stuffs - Isolation and enumeration of *Lactobacillus* spp.
- [13] *Supplementary information, Request for clock-stop, cs-fad-2017-0004-ProEquo.pdf, Ref. Ares(2017)5873157 - 30/11/2017
- [14] *Technical dossier, Section II: 2.6.2. Method for analyzing the active substance in feedingstuffs
- [15] *Technical dossier, Section II: Annex II-17 (Analyzis of active ingredients in feedingstuff)
- [16] *Technical dossier, Section II: 2.6.3. Identification
- [17] 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-2017-0004

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation is the “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

The following National Reference Laboratories contributed to this report:

- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Centre wallon de Recherches agronomiques (CRA-W), Gembloux (BE)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Univerza v Ljubljani. Veterinarska fakulteta. Nacionalni veterinarski inštitut. Enota za patologijo prehrane in higieno okolja, Ljubljana (SI)
- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, PESCA, Alimentació i Medi Natural. Generalitat de Catalunya, Cabrils (ES)