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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**

**Lactiplantibacillus plantarum DSM 11520**  
*(FEED-2021-2210; CRL/210056)*



**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: **FEED-2021-2210 - CRL/210056**

Name of Product: ***Lactiplantibacillus plantarum DSM 11520***

Active Agent (s): **Lactiplantibacillus plantarum**

Rapporteur Laboratory: **European Union Reference Laboratory for  
Feed Additives (EURL-FA)  
JRC Geel, Belgium**

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Date: **16/05/2022**

## EXECUTIVE SUMMARY

In the current application an authorisation is sought under Article 4 for a preparation of *Lactiplantibacillus plantarum* DSM 11520 under the category / functional group 1(j) 'technological additives' / 'acidity regulators' and 1(o) 'other technological additives' / 'starter cultures', according to Annex I of Regulation (EC) No 1831/2003. The authorisation is sought for the use of the *feed additive* for horses, dogs, cats and non-food producing rabbits (pets).

According to the Applicant, the *feed additive* contains as *active substance* viable cells of the non-genetically modified strain *Lactiplantibacillus plantarum* DSM 11520. The *feed additive* is to be marketed as a preparation with a minimum content of  $1.33 \times 10^{10}$  Colony Forming Unit (CFU) / g product. The product is intended to be added to feed materials of plant origin with high moisture content, including liquid *feedingstuffs*, with a minimum proposed dose of the *active substance* of  $8.0 \times 10^{10}$  CFU / kg feed material.

For the enumeration of *Lactiplantibacillus plantarum* DSM 11520 in the *feed additive* and *feedingstuffs* the Applicant presented for official control the ring-trial validated spread plate (or pour plate) method EN 15787.

Based on the performance characteristics, as already concluded for similar *Lactiplantibacillus* reports, the EURL recommends for official control the ring-trial validated EN 15787 method for the enumeration of *Lactiplantibacillus plantarum* DSM 11520 in the *feed additive* and *feedingstuffs*.

Furthermore, for the identification of *Lactiplantibacillus plantarum* DSM 11520, the EURL recommends for official control (i) DNA sequencing methods such as Whole Genome Sequencing (WGS) or (ii) Pulsed-Field Gel Electrophoresis (PFGE). The EURL considers that both methodologies are fit-for-purpose for the bacterial identification of authorised additives at a strain level.

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

*Lactiplantibacillus plantarum* DSM 11520, technological additives, acidity regulators, other technological additives, starter cultures, horses, dogs, cats and non-food producing rabbits (pets).

## 1. BACKGROUND

In the current application an authorisation is sought under Article 4(1) (new feed additive) for a preparation of *Lactiplantibacillus plantarum* DSM 11520 under the category / functional group 1(j) 'technological additives' / 'acidity regulators' and 1(o) 'other technological additives' / 'starter cultures', according to Annex I of Regulation (EC) No 1831/2003 [1-4]. The authorisation is sought for the use of the *feed additive* for horses, dogs, cats and non-food producing rabbits (pets) [4].

The *feed additive* is intended to be marketed as a dried powder preparation containing as active substance *Lactiplantibacillus plantarum* DSM 11520 [3,5]. The preparation contains viable cells of the microorganism with a minimum content of  $1.33 \times 10^{10}$  Colony Forming Unit (CFU) / g product [6].

The *Lactiplantibacillus plantarum* DSM 11520 is a non-genetically modified strain. The microorganism is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) under the accession number DSM 11520 [7].

The *feed additive* is intended to be used directly into feed materials of plant origin with high moisture content, including liquid *feedingstuffs*, with a minimum proposed dose of the active substance of  $8.0 \times 10^{10}$  CFU / kg *feed material* [5,8].

## 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 *Lactiplantibacillus plantarum* DSM 11520 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 *Lactiplantibacillus plantarum* in the *feed additive* and *feedingstuffs* the Applicant proposed the ring-trial validated EN 15787 method, which was recently revised by CEN resulting in updated method dedicated for the enumeration of lactobacilli spp. in

*feedingstuffs* (additives, *premixtures* and compound feeds excluding mineral feeds) that contain lactobacilli as a single microorganism component or in a mixture with other microorganisms [9,10].

Following the updated method's protocol, the sample (5 to 50 g) is suspended in phosphate buffered saline containing Polysorbate 80 (Tween® 80) (tPBS). For serial dilutions, the peptone salt solution (PSS) is used. The appropriate dilutions are then mixed on Petri plates using spread plate (or pour plate) methods with MRS (de Man, Rogosa, Sharp) agar. Alternatively, the MRS agar can be acidified or include triphenyl tetrazolium chloride (TTC). However, for routine purposes the non-modified MRS agar is an appropriate medium. The agar plates are incubated anaerobically at 37 °C for 48 to 72 h [10].

The following performance characteristics, expressed in terms of precision, are reported in the frame of the ring-trial validation studies after logarithmic transformation of the CFU values ranging from 7.40 to 8.03 log<sub>10</sub> CFU/g: a standard deviation for *repeatability* (S<sub>r</sub>) ranging from 0.10 to 0.26 log<sub>10</sub> CFU/g and a standard deviation for *reproducibility* (S<sub>R</sub>) ranging from 0.18 to 0.39 log<sub>10</sub> CFU/g [10].

In addition, a limit of quantification (LOQ) of 3x10<sup>3</sup> CFU/g can be derived using the considerations of EN ISO 7218 standard [11].

Furthermore, the Applicant successfully performed experiments demonstrating the suitability of the CEN method in the *feed additive* preparation (*Lactiplantibacillus plantarum* DSM 11520 in maltodextrin/oat bran carrier) [12].

Based on the performance characteristics and the available information, the EURL recommends for official control the ring-trial validated EN 15787 method for the enumeration of *Lactiplantibacillus plantarum* DSM 11520 in the *feed additive* and *feedingstuffs*.

***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 taxonomic identification of *Lactiplantibacillus plantarum* DSM 11520, the Applicant applied a DNA sequencing method such as Whole Genome Sequencing (WGS) [7].

In former reports for similar dossiers, the EURL recommended for official control Pulsed-Field Gel Electrophoresis (PFGE), a generally recognised methodology for the genetic identification of bacterial strains [13]. The method has been recently ring trial validated and is supposed to become a CEN Technical specification [14].

The EURL considers that the both methodologies are fit-for-purpose for the bacterial identification of authorised additives at a strain level.

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 (i) DNA sequencing methods such as Whole Genome Sequencing (WGS) or Pulsed-Field Gel Electrophoresis (PFGE) for the identification of *Lactiplantibacillus plantarum* DSM 11520; and (ii) the ring-trial validated spread plate (or pour plate) CEN method (EN 15787) for the enumeration of *Lactiplantibacillus plantarum* DSM 11520 in the *feed additive* and *feedingstuffs*.

##### ***Recommended text for the register entry (analytical method)***

- Identification: DNA sequencing methods or Pulsed-Field Gel Electrophoresis (PFGE)
- Enumeration in the *feed additive* and *feedingstuffs*: Spread plate (or pour 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 *Lactiplantibacillus plantarum* DSM 11520 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] Forwarding of applications for authorisation of feed additives in accordance with Regulation (EC) No 1831/2003 – E-Submission Food Chain platform – <https://webgate.ec.europa.eu/esfc/#/applications/2944>  
<https://open.efsa.europa.eu/questions/EFSA-Q-2021-00687>
- [2] \*Application, Annex 1
- [3] \*Technical dossier, Section II: 2.1.1. Name of the additive
- [4] \*Technical dossier, Section II: 2.1.2. Proposal for classification
- [5] \*Scientific Summary, Section II: 1. Introduction
- [6] \*Technical dossier, Section II: 2.1.3. Qualitative and quantitative composition
- [7] \*Technical dossier, Section II: 2.2.2.2. Micro-organisms

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- [8] \*Technical dossier, Section II: 2.5.1. Proposed mode of use in animal nutrition
- [9] \*Scientific Summary, Section II: 2.6.1. Methods of analysis for the active substance
- [10] EN 15787:2021 – Animal feeding stuffs: Methods of sampling and analysis - Detection and enumeration of *Lactobacillus* spp. used as feed additive
- [11] ISO 7218:2007 – Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations
- [12] \*Technical dossier, Section II: Annex II.6.1.1
- [13] EURL reports: [https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives/eurl-fa-authorisation/eurl-fa-evaluation-reports\\_en](https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives/eurl-fa-authorisation/eurl-fa-evaluation-reports_en)
- [14] CEN project TC 327 WI00327127 (2020): DNA fingerprinting of lactobacilli, pediococci, enterococci and bacilli in animal feeds by pulsed field gel electrophoresis (PFGE) Draft Report of a validation trial
- \*Refers to Dossier no: FEED-2021-2210

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

The Rapporteur Laboratory for this evaluation is the European Union Reference Laboratory for Feed Additives, JRC, Geel, 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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- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, PESCA, Alimentació i Medi Natural. Generalitat de Catalunya, Cabrils (ES)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)