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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 TAK 59 NCIMB 42150**  
*(FAD-2015-0013; CRL/150002)*





**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-2015-0013 – CRL/150002**

Name of Product: **Lactobacillus plantarum TAK 59 NCIMB  
42150**

Active Agent (s): ***Lactobacillus plantarum* TAK 59 NCIMB  
42150**

Rapporteur Laboratory: **Centre wallon de Recherches  
agronomiques (CRA-W), Gembloux,  
Belgium**

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Date: **22/10/2015**

Report approved by: **Christoph von Holst**  
Date: **22/10/2015**

## EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4(1) for *Lactobacillus plantarum* TAK 59 NCIMB 42150 under the category / functional group 1(k) 'technological additives' / 'silage additives', according to Annex I of Regulation (EC) No 1831/2003. Authorization is sought for the use of the *feed additive* for all animal species.

According to the Applicant, the *feed additive* contains as active substance viable cells of the non-genetically modified strain *Lactobacillus plantarum* TAK 59 NCIMB 42150. The *feed additive* is to be marketed as a powder containing a minimum *Lactobacillus plantarum* TAK 59 NCIMB 42150 concentration of  $1 \times 10^{11}$  Colony Forming Unit (CFU)/g. The *feed additive* is intended to be added to *silage* at a minimum dose of  $1 \times 10^5$  CFU/g fresh *silage*.

For the identification of *Lactobacillus plantarum* TAK 59 NCIMB 42150, the EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised standard methodology for genetic identification. This standard methodology for microbial identification is currently being evaluated by the CEN Technical Committee 327 to become a European Standard.

For enumeration of *Lactobacillus plantarum* TAK 59 NCIMB 42150, 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* TAK 59 NCIMB 42150 in the *feed additive per se*.

The Applicant did not provide any experimental method or data for the determination of *Lactobacillus plantarum* TAK 59 NCIMB 42150 in *silage*. Furthermore, the unambiguous determination of the content of *Lactobacillus plantarum* TAK 59 NCIMB 42150 added to *silage* is not achievable by analysis. Therefore, the EURL cannot evaluate nor recommend any method for official control to determine *Lactobacillus plantarum* TAK 59 NCIMB 42150 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* TAK 59 NCIMB 42150, technological additives, silage additives, all species.

## 1. BACKGROUND

In the current application authorisation is sought under Article 4(1) for *Lactobacillus plantarum* TAK 59 NCIMB 42150 under the category / functional group 1(k) 'technological additives' / 'silage additives', according to Annex I of Regulation (EC) No 1831/2003 [1]. Authorization 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* TAK 59 NCIMB 42150. The strain is deposited at NCIMB Ltd. (Scotland, UK) [2, 3].

The *feed additive* is to be marketed as a powder containing a minimum *Lactobacillus plantarum* TAK 59 NCIMB 42150 concentration of  $1 \times 10^{11}$  Colony Forming Unit (CFU)/g.

The *feed additive* is intended to be added to *silage* via a water suspension at a minimum dose of  $1 \times 10^5$  CFU/g fresh *silage* [2, 4].

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 [5].

## 2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009, 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* TAK 59 NCIMB 42150 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* TAK 59 NCIMB 42150, the Applicant used API characterization, 16S rRNA gene sequence analysis and enterobacterial repetitive intergenic consensus (ERIC)-PCR [3].

The EURL recommends instead for official control the PFGE, a generally recognised standard methodology for genetic identification [6]. 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 additive*

The Applicant analysed the *feed additive* for microbial contaminants (e.g. *Escherichia coli*, *Enterobacteriaceae* and *Salmonella*) using the methods described in the technical dossier [7]. As for the determination of other undesirable substances in the *feed additive* (e.g. arsenic, cadmium, lead, mercury, mycotoxins), analytical methods for official control are available from the respective European Union Reference Laboratories [8].

#### *Description of the analytical methods for the determination of the active substances in feed additive and silage*

For enumeration of *Lactobacillus plantarum* TAK 59 NCIMB 42150 in *feed additive*, the Applicant submitted the ring-trial validated spread plate method EN 15787 developed by CEN [3,9].

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 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) [9]:

- a standard deviation for repeatability (Sr) of 0.24 log<sub>10</sub> CFU/g;
- a standard deviation for reproducibility (SR) ranging from 0.29 to 0.38 log<sub>10</sub> CFU/g; and
- a limit of quantification (LOQ) of 3x10<sup>6</sup> CFU/kg of *feedingstuffs*, below the minimum dose of 1x10<sup>11</sup> CFU/kg *feedingstuffs* proposed by the Applicant.

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* TAK 59 NCIMB 42150 in *feed additive per se*.

The Applicant did not provide any experimental method or data for the determination of *Lactobacillus plantarum* TAK 59 NCIMB 42150 in *silage*. Furthermore, the unambiguous determination of the content of *Lactobacillus plantarum* TAK 59 NCIMB 42150 added to *silage* is not achievable by analysis. Therefore, the EURL cannot evaluate nor recommend

any method for official control to determine *Lactobacillus plantarum* TAK 59 NCIMB 42150 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* TAK 59 NCIMB 42150 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* TAK 59 NCIMB 42150 in *silage*. Furthermore, the unambiguous determination of the content of *Lactobacillus plantarum* TAK 59 NCIMB 42150 added to *silage* is not achievable by analysis. Therefore, the EURL cannot evaluate nor recommend any method for official control to determine *Lactobacillus plantarum* TAK 59 NCIMB 42150 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* TAK 59 NCIMB 42150 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 G1: F.A. 1831/0013-2015
- [2] \*Application, Proposal for Register Entry, Annex A
- [3] \*Technical dossier, Section II, Identity, characterisation and conditions of use of the additive
- [4] \* Technical dossier, Section I, Public summary

[5] 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>

[6] 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)

[7] \*Technical dossier, Section I - Annex\_II\_4\_Purity

[8] 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

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

\*Refers to Dossier no: FAD-2015-0013

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

The Rapporteur Laboratory for this evaluation was 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 (EC) No 885/2009.

## **8. ACKNOWLEDGEMENTS**

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- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Laboratoire de Rennes (SCL L35), Service Commun des Laboratoires DGCCRF et DGDDI, Rennes (FR)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)