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**EURL Evaluation Report on the Analytical Methods  
submitted in connection with the Application for the  
Authorisation of Feed Additives according to  
Regulation (EC) No 1831/2003**

**Dossier related to:** FAD-2011-0004  
CRL/100360

**Product Name:** *Lactobacillus plantarum E-98 NCIMB 30236*

**Active Substance(s):** *Lactobacillus plantarum E-98 NCIMB 30236*

**Rapporteur Laboratory:** European Union Reference Laboratory  
for Feed Additives (EURL-FA)  
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**Date:** 06/05/2011

## EXECUTIVE SUMMARY

In the current application authorisation is sought for the feed additive *Lactobacillus plantarum E-98 NCIMB 30236* under the category 'technological additives', functional group 'silage additives' according to Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the *feed additive* to be placed on the market in the form of powder, containing a minimum concentration of  $1.2 \times 10^{11}$  CFU/g of *Lactobacillus plantarum E-98 NCIMB 30236*. The intended use of the current application is for all animal species. It is proposed to be mixed into *silage* providing a minimum concentration of  $2.4 \times 10^5$  CFU/g fresh matter ensilage.

For enumeration of *Lactobacillus plantarum E-98 NCIMB 30236* in *feed additive*, the Applicant proposes internationally recognised, ring trial validated spread plate method developed by CEN (EN 15787). The performance characteristics reported after logarithmic transformation of measured values (CFU) are:

- a standard deviation for repeatability ( $S_r$ ) of  $0.24 \log_{10}$  CFU/g;
- a standard deviation for reproducibility ( $S_R$ ) ranging from  $0.29$  to  $0.38 \log_{10}$  CFU/g;
- and
- a limit of detection (LOD) of  $10^5$  CFU/kg *feedingstuffs*.

Based on the performance characteristics of the method the EURL recommends for official control the EN 15787 method for the determination of *Lactobacillus plantarum E-98 NCIMB 30236* in the *feed additive per se*.

The Applicant did not provide any experimental method or data for the determination of *Lactobacillus plantarum E-98 NCIMB 30236* in *silage*. Furthermore, the unambiguous determination of the content of *Lactobacillus plantarum E-98 NCIMB 30236* added to *silage* is not achievable by analysis. Therefore the EURL cannot evaluate nor recommend any method for official control to determine *Lactobacillus plantarum E-98 NCIMB 30236* in *silage*.

Molecular methods were used by the Applicant for identification of the active agent in the *feed additive*. The EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised standard methodology for microbial identification.

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* E-98 NCIMB 30236, technological additives, silage additives, all animal species.

## 1. BACKGROUND

*Lactobacillus plantarum* E-98 NCIMB 30236 is a feed additive for which authorisation under Article 4(1) is sought under the category of 'technological additives' functional group 'silage additives' according to Annex I of Regulation (EC) No 1831/2003 [1]. Specifically, authorisation is sought for the *feed additive* to be placed on the market as a powder, containing minimum concentration of  $1.2 \times 10^{11}$  CFU/g of *Lactobacillus plantarum* E-98 NCIMB 30236 [2]. *Lactobacillus plantarum* E-98 NCIMB 30236 is deposited in the National Collection of Industrial Bacteria (NCIMB) [3]. The intended use of the current application is for all animal species. It is proposed to be mixed into silage with water providing a minimum concentration of  $2.4 \times 10^5$  CFU/g [2, 4].

## 2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005 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 tasks of the European Union Reference Laboratory concerning applications for authorizations of *feed additives*, as last amended by Regulation (EC) No 885/2009, the EURL is requested to submit a full evaluation report to the European Food Safety Authority (EFSA) for each application, or for each group of applications. For this particular dossier, the methods of analysis submitted in connection with the *Lactobacillus plantarum* E-98 NCIMB 30236 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*

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

For identification and characterization of the strain *Lactobacillus plantarum* E-98 NCIMB 30236 the Applicant used Dot Blot hybridization with subspecies specific oligonucleotide probes (16S ribosomal RNA) and API characterization [5]. This method is suitable for the purpose of analysis. However, the EURL recommends for official control Pulsed Field Gel

Electrophoresis (PFGE), a generally recognised standard methodology for microbial identification [6].

*Qualitative and quantitative composition of impurities in the additive*

The Applicant analysed the *feed additive* for microbial contaminants (such as Enterobacteria, *Escherichia coli*, *Salmonella* spp.) by using appropriate EN ISO tests [7]. For undesirable substances (i.e. arsenic, cadmium, mercury, lead, selenium, copper, zinc, chrome, aflatoxins) internationally recognised standard methods are available at the respective European Union Reference Laboratory, in accordance with Commission Regulation (EC) No 776/2006.

***Description of the analytical methods for the determination of active substance in feed additive, premixtures and feedingstuffs***

For enumeration of *Lactobacillus plantarum* E-98 NCIMB 30236 in *feed additive*, the Applicant proposes internationally recognised, ring trial validated spread plate method developed by CEN (EN 15787) [8]. 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. The performance characteristics of the EN 15787 method reported after logarithmic transformation of measured values (CFU) are [8]:

- 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;
- and
- a limit of detection (LOD) of 10<sup>5</sup> CFU/kg of *feedingstuffs* [9].

Based on the performances characteristics of the method the EURL recommends for official control the EN 15787 for the determination of *Lactobacillus plantarum* E-98 NCIMB 30236 in the *feed additive per se*.

The Applicant did not provide any experimental method or data for the determination of *Lactobacillus plantarum* E-98 NCIMB 30236 in *silage*. Furthermore, the unambiguous determination of the content of *Lactobacillus plantarum* E-98 NCIMB 30236 added to *silage* is not achievable by analysis. Therefore the EURL cannot evaluate nor recommend any method for official control to determine *Lactobacillus plantarum* E-98 NCIMB 30236 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 the CEN method " EN 15787 " for the enumeration of *Lactobacillus plantarum* E-98 NCIMB 30236 in the *feed additive*. The EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE) for the identification of *Lactobacillus plantarum* E-98 NCIMB 3026.

The Applicant did not provide any experimental method or data for the determination of *Lactobacillus plantarum* E-98 NCIMB 30236 in *silage*. Furthermore, the unambiguous determination of the content of *Lactobacillus plantarum* E-98 NCIMB 30236 added to *silage* is not achievable by analysis. Therefore the EURL cannot evaluate nor recommend any method for official control to determine *Lactobacillus plantarum* E-98 NCIMB 30236 in *silage*.

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

- Enumeration in the *feed additive*: Spread plate method (EN 15787)
- Identification: Pulsed Field Gel Electrophoresis (PFGE)

#### **5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL**

In accordance with the requirements of Regulation (EC) No 1831/2003, samples of the additive *Lactobacillus plantarum* E-98 NCIMB 30236 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/Ref: SANCO/D/2: Forw.Appl.1831/(004)(10527)-2011
- [2] \*Application, Annex A, Proposal for register entry
- [3] \*Technical Dossier, Annex\_II\_1-\_ Certificate of deposition
- [4] \*Technical Dossier, Section II.2.5.1. Conditions of use
- [5] \*Technical Dossier, Section II.2.2 Characterisation of the active substance(s)/agent(s)
- [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)"
- [7] \*Technical Dossier, Section II.2.1.4. Purity
- [8] EN 15787 : " Animal feeding stuffs- Isolation and enumeration of *Lactobacillus* spp."
- [9] ISO 7218:1996, Microbiology of food and animal feedingstuffs – General rules for microbiological examinations

\*Refers to Dossier No: FAD-2011-0004

## 7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was European Union Reference Laboratory for Feed Additives, IRMM, 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 (EC) No 885/2009.

## 8. ACKNOWLEDGEMENTS

The following National Reference Laboratories contributed to this report:

- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Italy
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Czech Republic
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Austria
- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, France
- Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFÄ), Germany