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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 hilgardii CNCM I-4785

(FAD-2016-0050; CRL/160021)

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-2016-0050 – CRL/160021**
Name of Product:	***Lactobacillus hilgardii CNCM I-4785***
Active Agent (s):	**Lactobacillus hilgardii CNCM I-4785**
Rapporteur Laboratory:	**Centre wallon de Recherches agronomiques (CRA-W), Gembloux, Belgium**
Report prepared by:	**Véronique Ninane**
Report checked by: Date:	**Stefano Bellorini & Piotr Robouch (EURL-FA) 07/03/2017**
Report approved by: Date:	**Christoph von Holst 10/03/2017**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4(1) for *Lactobacillus hilgardii CNCM I-4785* 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* for all animal species.

According to the Applicant, the *feed additive* contains as *active substance* viable cells of the non-genetically modified strain *Lactobacillus hilgardii* CNCM I-4785. The *feed additive* is to be marketed as a powder containing a minimum *Lactobacillus hilgardii* CNCM I-4785 content of $3x10^{10}$ Colony Forming Unit (CFU)/g. The *feed additive* is intended to be added to *silage* at a minimum dose of $1.5x10^{8}$ CFU/kg fresh *silage.*

For the identification of *Lactobacillus hilgardii* CNCM I-4785, the EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised methodology for genetic identification of bacterial strains.

For the enumeration of *Lactobacillus hilgardii* CNCM I-4785 in the *feed additive per se*, 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.

Since the enumeration of initially added *Lactobacillus hilgardii* CNCM I-4785 in *silage* is not achievable by analysis, the EURL cannot recommend any method for official control.

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 hilgardii CNCM I-4785, technological additives, silage additives, all species.

1. BACKGROUND

In the current application authorisation is sought under Article 4(1) for *Lactobacillus hilgardii CNCM I-4785* 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* 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 hilgardii* CNCM I-4785. The strain is deposited at the Collection Nationale de Cultures de Micro-organismes (CNCM, Institut Pasteur, Paris) [3].

The *feed additive* has a minimum *Lactobacillus hilgardii* CNCM I-4785 content of $3x10^{10}$ Colony Forming Unit (CFU)/g [2]. The *feed additive* is intended to be marketed as a powder further formulated with other technological feed additives [3].

The *feed additive* is intended to be added directly to *silage* at a minimum dose of $1.5x10^{8}$ CFU/kg fresh *silage* in combination with an equal amount of Lactobacillus buchneri NCIMB 40788 CNCM I-4323 [2,4-6].

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 hilgardii CNCM I-4785* 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 hilgardii CNCM I-4785*, the Applicant applied 16S rRNA partial gene sequence analysis and pulsed-field gel electrophoresis (PFGE) of restricted DNA [4].

The EURL recommends for official control the PFGE, a generally recognised standard methodology for genetic identification [7]. This methodology for microbial identification is currently being evaluated by the CEN Technical Committee 327 to become European Standard.

Qualitative and quantitative composition of impurities in the additive

The Applicant analysed the *feed additive* for microbial contaminants (e.g. yeast and mould *Escherichia coli*, staphylococci, coliforms and *Salmonella*) using the methods mentioned in the technical dossier [3]. As for the determination of other undesirable substances in the *feed additive* (e.g. 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 the enumeration of *Lactobacillus hilgardii* CNCM I-4785 in the *feed additive* and in a "mixtures of silage additives", the Applicant submitted the ring-trial validated spread plate method EN 15787 developed by CEN for the analysis of *Lactobacillus* spp. [2,4,9].

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 anaerobically at 37 °C for 48 to 72 hours. The following performance characteristics were reported after logarithmic transformation of the CFU values [9]:

- 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, according to ISO 7218, the EURL calculated a limit of quantification (LOQ) of $3x10^3$ CFU/g [10].

Based on the performance characteristics presented, the EURL recommends for official control the ring-trial validated EN 15787 method for the enumeration of *Lactobacillus hilgardii* CNCM I-4785 in *feed additive per se*.

Since the unambiguous determination of *Lactobacillus hilgardii* CNCM I-4785 added to *silage* is not achievable by analysis, the EURL cannot recommend the EN 15787 or any other method for official control to quantify the microorganism of concern 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 hilgardii* CNCM I-4785 and the ring-trial validated spread plate method EN 15787 for enumeration of this strain in the *feed additive*.

Since the unambiguous determination of *Lactobacillus hilgardii* CNCM I-4785 added to *silage* is not achievable by analysis, the EURL cannot recommend the EN 15787 or any other method for official control to quantify the micro-organism of concern 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 hilgardii CNCM I-4785* 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. 1831/0042-2016
[2] *Suppl. Information: Proposed registry entry updated.doc
[3] *Technical dossier, Section II, 2.1.3.1. Qualitative composition of the additive
[4] *Technical dossier, Section II, Identity, characterisation and conditions of use of the additive
[5] *Technical dossier, Section I, Public summary
[6] Commission Implementing Regulation (EU) No 1113/2013 concerning the authorisation of preparations of Lactobacillus plantarum NCIMB 40027, Lactobacillus buchneri DSM 22501, Lactobacillus buchneri NCIMB 40788/CNCM I-4323, Lactobacillus buchneri LN 40177/ATCC PTA-6138, and Lactobacillus buchneri LN 4637/ATCC PTA-2494 as feed additives for all animal species
[7] 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)
[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.
[10] EN ISO 7218:2007 - Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations

*Refers to Dossier no: FAD-2016-0050

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

The following National Reference Laboratories contributed to this report:

- Centro di referenza nazionale per la sorveglienza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Instytut Zootechniki — Państwowy Instytut Badawczy, Krajowe Laboratorium Pasz, Lublin (PL)
- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, PESCA, Alimentació i Medi Natural. Generalitat de Catalunya, Cabrils (ES)
- Laboratoire de Rennes (SCL L35), Service Commun des Laboratoires DGCCRF et DGDDI, Rennes (FR)