



JRC.D.5/FSQ/CvH/DM/ag/ARES(2012)253836

**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-2010-0255**
CRL/100329

Name of Feed Additive: ***Propionibacterium acidipropionici***
CNCM MA 26/4U

Active Substance(s): ***Propionibacterium acidipropionici***
CNCM MA 26/4U

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

Report prepared by: **Dijana Mitić (EURL-FA)**

Report revised by: **Piotr Robouch (EURL-FA)**
Date: **05/03/2012**

Report approved by: **Christoph von Holst**
Date: **05/03/2012**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 10(7) for *Propionibacterium acidipropionici* CNCM MA 26/4U, under the category/functional group 1(k), "technological additives/silage additives", according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the *feed additive* for all animal species and categories. The *feed additive* is to be placed on the market as a powder, containing a minimum concentration of 1×10^{10} CFU/g of *Propionibacterium acidipropionici* CNCM MA 26/4U. It is intended to be mixed directly into silage or suspended in water and sprayed with a minimum concentration of 1×10^8 CFU/kg *fresh forage*.

For enumeration of *Propionibacterium acidipropionici* CNCM MA 26/4U in *feed additive*, the Applicant submitted the ring-trial validated spread plate method developed for the enumeration of *Lactobacillus* spp (EN 15787). The Applicant applied, upon the request by the EURL, the above mentioned method to the enumeration of *Propionibacterium acidipropionici*. The following performance characteristics were recalculated by EURL, based on the experimental data provided by the Applicant:

- a standard deviation for *repeatability* (S_r) of $0.09 \log_{10}$ CFU/g;
- a standard deviation for *intermediate precision* (S_{ip}) of $0.10 \log_{10}$ CFU/g; and
- a limit of detection (LOD) of 10^5 CFU/kg *feedingstuffs*.

Based on the performances characteristics presented the EURL recommends for official control the CEN method (EN 15787) for the determination of *Propionibacterium acidipropionici* CNCM MA 26/4U in the *feed additive per se*.

The Applicant did not provide any experimental method or data for the determination of *Propionibacterium acidipropionici* CNCM MA 26/4U in *silage*. Furthermore, the unambiguous determination of the content of *Propionibacterium acidipropionici* CNCM MA 26/4U added to *silage* is not achievable by analysis. Therefore the EURL cannot evaluate nor recommend any method for official control to determine *Propionibacterium acidipropionici* CNCM MA 26/4U in *silage*.

Molecular methods were used by the Applicant to identify the active agent in the *feed additive*. The EURL recommends instead 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

Propionibacterium acidipropionici CNCM MA 26/4U, technological additives, silage additives, all animal species and categories.

1. BACKGROUND

In the current application authorisation is sought under Article 10(7) (re-evaluation of already authorised additives) for *Propionibacterium acidipropionici* CNCM MA 26/4U, under the category/functional group 1(k), "technological additives/silage additives", according to the classification system of Annex I of Regulation (EC) No 1831/2003 [1]. Specifically, authorisation is sought for the use of the *feed additive* for all animal species and categories. The *feed additive* is to be placed on the market as a powder, containing a minimum concentration of 1×10^{10} CFU/g of *Propionibacterium acidipropionici* CNCM MA 26/4U [2]. *Propionibacterium acidipropionici* CNCM MA 26/4U is deposited in the 'Collection Nationale De Cultures De Microorganismes (CNCM)' in Paris, France [3]. It is intended to be mixed directly into silage or suspended in water and sprayed with a minimum concentration of 1×10^8 CFU/kg *fresh forage* [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 authorisations 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 *Propionibacterium acidipropionici* CNCM MA 26/4U 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 characterisation of the strain *Propionibacterium acidipropionici* CNCM MA 26/4U the Applicant used Pulsed Field Gel Electrophoresis (PFGE) and API characterisation [5]. These methods are suitable for the purpose of analysis. The EURL

recommends instead 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 coliforms, *Escherichia coli*, *Salmonella* spp., yeasts and moulds) 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 *Propionibacterium acidipropionici* CNCM MA 26/4U in *feed additive*, the Applicant proposed the ring-trial validated spread plate method (EN 15787) [8], developed for the enumeration of *Lactobacillus* spp. The sample is suspended and diluted in a phosphate buffered saline (PBS); 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 CEN method reported after logarithmic transformation are [8]:

- a standard deviation for *repeatability* (S_r) of 0.24 log₁₀ CFU/g;
- a standard deviation for *reproducibility* (S_R) ranging from 0.29 to 0.38 log₁₀ CFU/g; and
- a limit of detection (LOD) of 10⁵ CFU/kg of *feedingstuffs* [9].

The Applicant applied, upon the request by the EURL, the above mentioned method to the enumeration of *Propionibacterium acidipropionici* [10]. The following performance characteristics were recalculated by EURL [11], based on the experimental data provided by the Applicant:

- $S_r = 0.09$ log₁₀ CFU/g; and
- a standard deviation for *intermediate precision* (S_{ip}) of 0.10 log₁₀ CFU/g.

These performance characteristics are in good agreement with those reported in the CEN standard. Therefore, the Applicant demonstrated the applicability of the CEN method to the enumeration of *Propionibacterium acidipropionici*.

Based on the performances characteristics presented the EURL finds the method suitable for the determination of *Propionibacterium acidipropionici* and recommends for official control the CEN method (EN 15787) for the determination of *Propionibacterium acidipropionici* CNCM MA 26/4U in the *feed additive per se*.

The Applicant did not provide any experimental method or data for the determination of *Propionibacterium acidipropionici* CNCM MA 26/4U in *silage*. Furthermore, the unambiguous determination of the content of *Propionibacterium acidipropionici* CNCM MA 26/4U added to *silage* is not achievable by analysis. Therefore the EURL cannot evaluate nor recommend any method for official control to determine *Propionibacterium acidipropionici* CNCM MA 26/4U 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 *Propionibacterium acidipropionici* CNCM MA 26/4U in the *feed additive* and Pulsed Field Gel Electrophoresis (PFGE) for its identification.

The Applicant did not provide any experimental method or data for the determination of *Propionibacterium acidipropionici* CNCM MA 26/4U in *silage*. Furthermore, the unambiguous determination of the content of *Propionibacterium acidipropionici* CNCM MA 26/4U added to *silage* is not achievable by analysis. Therefore the EURL cannot evaluate nor recommend any method for official control to determine *Propionibacterium acidipropionici* CNCM MA 26/4U in *silage*.

Recommended text for the register entry (analytical method)

- Enumeration of *Propionibacterium acidipropionici* CNCM MA 26/4U in the *feed additive*: Spread plate method (EN 15787)
- Identification of *Propionibacterium acidipropionici* CNCM MA 26/4U: 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 *Propionibacterium acidipropionici* CNCM MA 26/4U 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/G1: Forw.Appl.1831/0025-2011
- [2] *Application, Annex A, Proposal for register entry
- [3] *Technical Dossier, Section II, Annex II_2.2.1.2.2
- [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 15787:2009 - Animal feeding stuffs- Isolation and enumeration of *Lactobacillus* spp.
- [9] ISO 7218:1996 - Microbiology of food and animal feedingstuffs – General rules for microbiological examinations
- [10] *Supplementary Information, Additional Info Verification 13-01-2012
- [11] *Supplementary Information, EURL *Propionibacterium* verif
*Refers to Dossier No: FAD-2010-0255

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:

- Skúšobné laboratórium – Oddelenie analýzy krmív, Ústredný kontrolný a skúšobný ústav poľnohospodársky, Bratislava (SK)

- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen, Jena (DE)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Państwowy Instytut Weterynaryjny, Puławy (PL)
- Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim (DE)
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)