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DIRECTORATE GENERAL  
JOINT RESEARCH CENTRE  
Directorate D: Institute for Reference Materials and Measurements  
**European Union Reference Laboratory for Feed Additives**

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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**

***Pediococcus acidilactici* CNCM MA 18/5M  
(FAD-2013-0031; CRL/130021)**





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Dossier related to: **FAD-2013-0031 - CRL/130021**

Name of Product: **Bactocell PA 10 ME**

Active Agent (s): ***Pediococcus acidilactici* CNCM MA 18/5M**

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

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Report approved by: **Christoph von Holst**  
Date: **15/04/2014**

## EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4(1) for *Pediococcus acidilactici* CNCM MA 18/5M under the category/functional group 4(d) 'zootechnical additives' / 'other zootechnical additives', according to Annex I of Regulation (EC) No 1831/2003. Specifically, authorization is sought for the use of the *feed additive* for minor avian and minor porcine species. The feed additive is already authorised as 'zootechnical additives' / 'gut flora stabilisers' for piglets (weaned) under Commission Regulation (EU) No 1120/2010. The product is intended to be marketed as a micro-encapsulated white powder (Bactocell PA 10 ME) consisting of non-genetically modified viable bacterial strain of *Pediococcus acidilactici* CNCM MA 18/5M at a level of  $1 \times 10^{10}$  Colony Forming Units (CFU) per gram and 90 % anti-caking agent and carrier. The feed additive is to be used through *premixtures* in *feedingstuffs* and *water* at minimum doses of  $1 \times 10^9$  CFU/kg or  $5 \times 10^8$  CFU/L, respectively.

For the enumeration of *Pediococcus acidilactici* CNCM MA 18/5M in *feed additive*, *premixtures*, *feedingstuffs* and *water* the Applicant proposes the CEN spread plate method EN 15786:2009. The following performance characteristics were reported after logarithmic transformation (CFU):

- a repeatability standard deviation ( $s_r$ ) ranging from 0.01 to 0.17  $\log_{10}$  CFU/g,
- a reproducibility standard deviation ( $s_R$ ) ranging from 0.10 to 0.26  $\log_{10}$  CFU/g and
- a limit of detection (LOD) of  $1 \times 10^5$  CFU/kg, well below the minimum dose proposed by the Applicant.

Based on these performance characteristics, the EURL recommends, for official control, the CEN method EN 15786 for the enumeration of *Pediococcus acidilactici* CNCM MA 18/5M in *feed additive*, *premixtures*, *feedingstuffs* (excluding mineral feeds) and *water*.

Pulsed Field Gel Electrophoresis (PFGE) was used by the Applicant for identification and characterization of the active agent. This generally recognised standard methodology for microbial identification, is recommended by EURL 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

*Pediococcus acidilactici* CNCM MA 18/5M, zootechnical additives, other zootechnical additive, minor avian and minor porcine species.

## 1. BACKGROUND

In the current application authorisation is sought under Article 4(1) (new use of a feed additive already authorised) for *Pediococcus acidilactici* CNCM MA 18/5M under the category / functional group 4(d) 'zootechnical additives' / 'other zootechnical additives', according to Annex I of Regulation (EC) No 1831/2003 [1]. Specifically, authorization is sought for the use of the *feed additive* for minor avian and minor porcine species. The feed additive is already authorised as 'zootechnical additives' / 'gut flora stabilisers' for piglets (weaned) under Commission Regulation (EU) No 1120/2010.

The product is intended to be marketed as a micro-encapsulated white powder (Bactocell PA 10 ME) consisting of non-genetically modified viable bacterial strain of *Pediococcus acidilactici* CNCM MA 18/5M at a level of  $1 \times 10^{10}$  Colony Forming Units (CFU) per gram feed additive and 90 % anti-caking agent and carrier [2,3].

The feed additive is intended to be used through *premixtures* in *feedingstuffs* and *water* at minimum doses of  $1 \times 10^9$  CFU/kg or  $5 \times 10^8$  CFU/L, respectively [3, 4].

Note: The EURL already evaluated the methods of analysis for *Pediococcus acidilactici* CNCM MA 18/5M in the report FAD-2008-0015 and FAD-2009-0025 [5, 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 *Bactocell PA 10ME* 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 impurities in the additive*

When required by EU legislation, analytical methods for official control of undesirable substances in the *feed additive* (e.g. arsenic, cadmium, lead, mercury, aflatoxin B1 and dioxins) are available from the respective European Union Reference Laboratories [7].

Furthermore, the Applicant analysed the *feed additive* for microbial contaminants including pathogenic staphylococci, coliforms and Salmonella using the methods described in the technical dossier provided [8, 9].

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

For identification and characterization of the strain *Pediococcus acidilactici* CNCM MA 18/5M the Applicant used Pulsed Field Gel Electrophoresis (PFGE) [10]. The EURL recommends for official control this generally recognised standard methodology for genetic identification [11]. This standard methodology for microbial identification, is currently being evaluated by the CEN Technical Committee 327 to become a European Standard.

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

For enumeration of *Pediococcus acidilactici* CNCM MA 18/5M in *feed additive, premixtures, feedingstuffs* and *water* the Applicant proposes the CEN method - EN 15786:2009 - an internationally recognised spread plate method. The sample is suspended and diluted in a buffer solution; the appropriated dilutions are then spread on MRS (de Man, Rogosa, Sharp) agar plates. The agar plates are incubated at 37°C for 48 hours [12].

The following performance characteristics are reported after logarithmic transformation (CFU):

- a *repeatability* standard deviation ( $s_r$ ) ranging from 0.01 to 0.17  $\log_{10}$  CFU/g,
- a *reproducibility* standard deviation ( $s_R$ ) ranging from 0.10 to 0.26  $\log_{10}$  CFU/g and
- a limit of detection (LOD) of  $1 \times 10^5$  CFU/kg [13], well below the minimum doses proposed by the Applicant ( $1 \times 10^9$  CFU/kg *feedingstuffs* or  $1 \times 10^8$  CFU/L *water*).

Furthermore, the Applicant provided experimental evidence demonstrating the applicability of the CEN method for the enumeration of *Pediococcus acidilactici* CNCM MA 18/5M in the micro-encapsulated form and in water in the frame of the homogeneity and stability studies [14-16].

Based on the experimental data provided the EURL recommends, for official control, the CEN method (EN 15786:2009) for the enumeration of *Pediococcus acidilactici* CNCM MA 18/5M in the *feed additive, premixtures, feedingstuffs* (excluding mineral feeds) and *water*.

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 the CEN method - EN 15786:2009 - for the enumeration of the active agent *Pediococcus acidilactici* CNCM MA 18/5M in *feed additive, premixtures, feedingstuffs and water*.

For the identification of *Pediococcus acidilactici* CNCM MA 18/5M the EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE). This method is currently evaluated by the CEN technical Committee 327 to become a European Standard.

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

- Enumeration: Spread plate method using MRS agar - EN 15786:2009
- Identification: Pulsed Field Gel Electrophoresis (PFGE)

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

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Bactocell PA 10 ME* 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 SANCO/G1: Forw. Appl. 1831/0030-2013
- [2] \*Technical dossier, Section I, 1. Public summary
- [3] \*Application, Proposal for Register Entry, Annex A
- [4] \*Technical dossier, Section II, 5. Conditions of use of the additive
- [5] <sup>a</sup>FAD-2008-0015, Bactocell PA 10 or Fermaid PA 10, Ref. 08/FSQ/CVH/RL/D(2008)28496 – 04/11/2008.
- [6] <sup>b</sup>FAD-2009-0025, Bactocell PA 10, Ref. JRC.DDG.D.6/CvH/DM/mds/ARES (2010)139620 – 15/03/2010
- [7] 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
- [8] \*Technical dossier, Section II, 1.4. Purity
- [9] \*Technical dossier, Section II, 2. Characterisation of the active substance(s) and or agent(s)
- [10] \*Technical dossier, Section II, 6. Method of analysis and reference samples
- [11] 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).

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- [12] EN 15786:2009 - Animal feeding stuffs- Isolation and enumeration of *Pediococcus* spp.
- [13] ISO 7218:2007 - Microbiology of food and animal feedingstuffs – General requirements and guidance for microbiological examinations
- [14] \*Technical dossier, Section II, Annexes – Annex\_II\_4\_1b.
- [15] \*Technical dossier, Section II, Annexes – Annex\_II\_4\_4\_13.
- [16] \*Technical dossier, Section II, Annexes – Annex\_II\_4\_4\_14.
- \*Refers to Dossier no: FAD-2013-0031
- <sup>a</sup><http://irrm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD2008-0015.pdf>
- <sup>b</sup><http://irrm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD2009-0025.pdf>

## 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).
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- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen, Jena (DE).
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT).
- Państwowy Instytut Weterynaryjny, Puławy (PL).
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