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CRL Evaluation Report on the Analytical Methods submitted in connection with the Application for Authorisation as a Feed Additive according to Regulation (EC) No 1831/2003

Dossier related to: FAD-2009-0025
FAD-2009-0026
CRL/090011

Name of Additive: Bactocell PA 10

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

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EXECUTIVE SUMMARY

In the current application authorisation is sought for the microbial feed additive *Pediococcus acidilactici* CNCM MA 18/5M under the category 'zootechnical additives', functional group 'gut flora stabiliser' according to Annex I of Regulation (EC) No 1831/2003. Specifically, authorization is sought for the use of *Pediococcus acidilactici* CNCM MA 18/5M for laying hens (FAD-2009-0025) and for piglets (weaned) (FAD-2009-0026). The feed additive is presented in a powder form with a concentration of 1×10^{10} CFU/g. It is intended to be mixed at a dose ranging from 7.5×10^8 to 1×10^9 CFU/kg of *feedingstuffs* for laying hens and from 7.2×10^8 to 1.1×10^9 CFU/kg of *feedingstuffs* for piglets (weaned).

For the enumeration of *Pediococcus acidilactici* CNCM MA 18/5M in *feed additives*, *premixtures* and *feedingstuffs* the CRL recommends CEN method (EN 15786:2009). The performance characteristics of the CEN method reported after logarithmic transformation (CFU) are:

- 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 1×10^5 CFU/kg, well below the minimum dose proposed by the applicant (7.2×10^8 CFU/kg of *feedingstuffs*).

Molecular methods were used by the applicant for identification of the active agent. The CRL recommends for official control, Pulsed Field Gel Electrophoresis (PFGE), a generally recognised standard methodology for microbial identification. The CEN Technical Committee 327 is currently developing a European Standard for this methodology.

Further testing or validation is not considered necessary.

KEYWORDS

Pediococcus acidilactici CNCM MA 18/5M, zootechnical additives, laying hens, piglets (weaned), gut flora stabilisers.

1. BACKGROUND

Pediococcus acidilactici CNCM MA 18/5M is a feed additive for which authorisation under Article 4(1) is sought under the category of 'zootechnical additives' functional group 'gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003 [1, 2]. The strain is deposited at the 'Collection Nationale de Cultures de Micro-organismes (CNCM)' within the 'Pasteur Institute' in Paris, France [3]. Specifically, authorisation is sought for feed additive intended to be mixed at a dosage ranging from 7.5×10^8 to 1×10^9 CFU/kg of *feedingstuffs* for laying hens (FAD-2009-0025) [4] and from 7.2×10^8 to 1.1×10^9 CFU/kg of *feedingstuffs* for piglets (weaned) (FAD-2009-0026) [5].

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 Community Reference Laboratory concerning applications for authorizations of *feed additives*, as last amended by Regulation (EC) No 885/2009, the CRL 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 *Pediococcus acidilactici* CNCM MA 18/5M dossiers, FAD-2009-0025 [4] and FAD-2009-0026 [5], 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 *Pediococcus acidilactici* CNCM MA 18/5M the applicant used Pulsed Field Gel Electrophoresis (PFGE). This method is suitable for the purpose of analysis [6].

The CRL recommends for official control, PFGE, a generally recognised standard methodology for genetic identification. The CEN technical Committee 327 is currently developing a European Standard for this methodology.

Qualitative and quantitative composition of any impurities in the additive

The applicant analysed the *feed additive* for microbial contaminants (such as Enterobacteria, *Escherichia coli*, *Salmonella* and yeasts) by using appropriate EN ISO tests [7 - 9], as well as for pathogenic staphylococci, coliforms, and anaerobic sulfite reducing bacteria by using the methods described in the technical dossier provided by the applicant [10 - 12].

For undesirable substances (i.e. arsenic, cadmium, mercury, lead, selenium, copper, zinc, chrome, aflatoxins) internationally recognised standard methods are available at the respective Community Reference Laboratories, in accordance with COMMISSION REGULATION (EC) No 776/2006.

Description of the analytical methods for the determination of active agent(s) in feed additive, premixtures and feedingstuffs

For enumeration of *Pediococcus acidilactici* CNCM MA 18/5M in *feed additive, premixtures* and *feedingstuffs*, 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 [13]. The performance characteristics of the CEN method reported after logarithmic transformation (CFU) are:

- 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 1×10^5 CFU/kg [14], well below the minimum dose proposed by the applicant (7.2×10^8 CFU/kg of *feedingstuffs*).

The CRL recommends, for official control, CEN method (EN 15786:2009) for the enumeration of *Pediococcus acidilactici* CNCM MA 18/5M in *feed additives, premixtures* and *feedingstuffs*.

4. CONCLUSIONS AND RECOMMENDATIONS

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

For the analysis of the identity of the bacterial strain, *Pediococcus acidilactici* CNCM MA 18/5M the CRL recommends Pulsed Field Gel Electrophoresis (PFGE) for official control. The CEN technical Committee 327 is currently developing a European Standard for this methodology.

Further testing or validation is not considered necessary.

Recommended text for the register entry, fourth column (Composition, chemical formula, description, analytical method)

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

5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

In accordance with the requirements of Regulation (EC) No 1831/2003, samples of the additive *Pediococcus acidilactici* CNCM MA 18/5M for laying hens and piglets (weaned) have been sent to the Community Reference Laboratory for Feed Additives Authorisation. The dossier has been made available to the CRL by EFSA.

6. REFERENCES

- [1] *Application/Ref:SANCO/D/2:Forw.Appl.1831/020-2009 [FAD-2009-0025]
- [2] *Application/Ref:SANCO/D/2:Forw.Appl.1831/021-2009 [FAD-2009-0026]
- [3] *Technical dossier, section II/ 2.1.2. Micro-organisms
- [4] *Application, Proposal for Register Entry, Annex A [FAD-2009-0025]
- [5] *Application, Proposal for Register Entry, Annex A [FAD-2009-0026]
- [6] *Technical dossier, section II/ Annex_II_6_5
- [7] *Technical dossier, section II/ Annex_II_6_13
- [8] *Technical dossier, section II/ Annex_II_6_14
- [9] *Technical dossier, section II/ Annex_II_6_16
- [10] *Technical dossier, section II/Annex_II_6_11
- [11] *Technical dossier, section II/Annex_II_6_12
- [12] *Technical dossier, section II/Annex_II_6_15
- [13] EN 15789:2009 'Animal feeding stuffs–Isolation and enumeration of *Pediococcus* spp'
- [14] ISO 7218:2007 'Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations'

*Refers to Dossier no: FAD-2009-0025 and FAD 2009-0026

7. RAPPORTEUR LABORATORY

The Rapporteur Laboratory for this evaluation was Community 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

8. Acknowledgements

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- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes (FR)
- Instytut Zootechniki w Krakowie, Krajowe Laboratorium Pasz, Lublin (PL)