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CRL Evaluation Report on the Analytical Methods submitted in
connection with Section II, 2.5 (Control Methods) of the Application
for Authorisation as a Feed Additive
according to Regulation (EC) No 1831/2003

Dossier related to: EFSA-Q-2007-205
FAD-2007-0046

Name of Additive: Bactocell PA or Fermaid PA

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

Rapporteur Laboratory: Community Reference Laboratory for
Feed Additives (CRL-FA)

Report prepared by: Renata Leuschner (CRL-FA)

Report revised by: Christoph von Holst (CRL-FA)

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Report approved by: Christoph von Holst (CRL-FA)
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EXECUTIVE SUMMARY

In the current application authorisation is sought for Bactocell PA or Fermaid PA under the category 'zootechnical additives', functional group 'other zootechnical additives' according to Annex I of Regulation (EC) No 1831/2003. Specifically, the use of this product for fish is requested. The product contains *Pediococcus acidilactici* CNCM MA 18/5M as active agent at a concentration of 10^{10} viable cells c.f.u. (colony forming units) per gram. The feed additive can be effectively used in any feed for fish at a target dose of 1 to 3×10^9 c.f.u./kg complete feedingstuffs.

For the determination of the active agent (*Pediococcus acidilactici* CNCM MA 18/5M) in the feed additive, *premixtures* and *feedingstuffs*, the applicant proposes a ring-trial validated method. The method's performance characteristics are standard deviations for repeatability (s_r) and reproducibility (s_R) of around $0.13 - 0.17 \log_{10}$ and $0.20 - 0.26 \log_{10}$ calculated from the base 10 logarithms of the measured c.f.u./g in feedingstuffs, respectively [J. AOAC 2003, 86, 791-801]. This method is recommended for official controls. The spread plate method has a limit of quantification (LOQ) of 10×10^6 c.f.u./kg.

For identification of the active agents, methods suitable for the purpose of analysis were used by the applicant. For official controls pulsed-field gel electrophoresis (PFGE) is recommended for the field of application sought.

On the basis of the supplied documentation, no supplementary experimental work (testing or method validation) is required by the CRL.

KEYWORDS

Bactocell PA, Fermaid PA, feed additive, fish, zootechnical, *Pediococcus acidilactici*

1. BACKGROUND

Bactocell PA or Fermaid PA is a product for which authorisation is sought under the category 'zootechnical additives', functional group 'other zootechnical additives' according to Annex I of Regulation (EC) No 1831/2003. Bactocell PA contains a minimum of 1×10^{10} c.f.u. viable cells of the strain *Pediococcus acidilactici* CNCM MA 18/5M as the active agent per gram. The strain is deposited at the Collection Nationale de Cultures de Microorganismes (CNCM), Institut Pasteur, Paris, France. The intended use of the current application is for fish, by mixing the feed additive into feedingstuffs at a target dose of 1 to 3×10^9 c.f.u. per kg complete feedingstuffs [1, 2, 3].

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 authorisations of feed additives, the CRL is requested to submit a full evaluation report to the European Food Safety Authority (EFSA) for each application. For this particular dossier, the methods of analysis submitted in connection with the Bactocell PA or Fermaid PA dossier (EFSA-Q-2007-205) and their suitability to be used for official controls in the frame of the authorisation, were evaluated.

3. EVALUATION

The numbering system under this point refers to the 'Guidelines for the assessment of additives in feedingstuffs, part II: Enzymes and Micro-organisms' (2.5 Control methods), in the following referred to as 'the Guidelines'.

Description of some of the methods listed under item 2.5.1. of the Guidelines

Qualitative and quantitative composition of the additive

The method for quantifying the active agent in the additive as provided by the applicant represents a spread plate method using Man Rogosa Sharp (MRS) agar which is appropriate for official controls [4].

The strain *Pediococcus acidilactici* CNCM MA 18/5M is characterised by the applicant for its physiological, biochemical and molecular properties [5]. For molecular identification a molecular fingerprint is provided [6]. Pulsed-field gel electrophoresis (PFGE) is considered as a suitable method for official controls.

Qualitative and quantitative composition of any impurities in the additive

The applicant provided quality control results for contents of heavy metals including arsenic (As), cadmium (Cd), lead (Pb), mercury (Hg), selenium, aflatoxin B1 and dioxin and dioxin-like PCBs using appropriate methodologies [7]. Microbiological quality of the additive was ensured by examination for coliforms, *Escherichia coli*, pathogenic staphylococci, enterococci, yeast and moulds, anaerobic sulfite reducing bacteria and *Salmonella* species by using suitable methods [7]. For official controls internationally recognised International standard Organisation (ISO) and Committee for European Normation (CEN) standard methods where available are recommended in line with current European Community Regulations.

Description of qualitative and quantitative methods for routine control of the active agent in premixtures and feedingstuffs (cf. requirements of Guidelines section 2.5.2)

To analyse premixtures and feedingstuffs for the active agent of the product, the applicant proposes a ring trial validated spread plate method using Man Rogosa Sharp (MRS) agar which is appropriate for official controls [4].

The method's performance characteristics are standard deviations for repeatability (s_r) and reproducibility (s_R) of around 0.13 – 0.17 \log_{10} and 0.20 – 0.26 \log_{10} calculated from the base 10 logarithms of the measured c.f.u./g in feedingstuffs, respectively [J. AOAC 2003, 86, 791-801]. This method is recommended for official controls. The spread plate method has a limit of quantification (LOQ) of 10×10^6 c.f.u./kg.

For identification of the active agents, methods suitable for the purpose of analysis were used by the applicant. For official controls pulsed-field gel electrophoresis (PFGE) is recommended for the field of application sought.

4. CONCLUSIONS AND RECOMMENDATIONS

Concerning the enumeration of the active agent a ring trial validated spread plate method using Man Rogosa Sharp (MRS) agar is recommended for official controls in the frame of the authorisation [4]. Pulsed-field gel electrophoresis (PFGE) is widely used by reference laboratories to identify bacterial isolates and it is therefore recommended as a suitable methodology in this context for official controls.

On the basis of the supplied documentation, no supplementary experimental work (testing or method validation) is required.

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

Enumeration: Spread plate method using MRS agar and 37 °C as incubation temperature

Identification: Pulsed-field gel electrophoresis (PFGE) method

5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

In accordance with the requirements of Regulation (EC) No 1831/2003, samples of the additive Bactocell PA or Fermaid PA for fish have been sent to the Community Reference Laboratory for Feed Additives. The dossier has been made available to the CRL-FA by EFSA.

6. REFERENCES

- [1] Proposal of Register entry, Annex III
- [2] Technical dossier, Section I, Public summary, 1.4. Proposed classification and identification of the feed additive
- [3] Technical dossier, Section VI, Form of identification note, VI. 2 Specifications concerning the active agent
- [4] Leuschner R.G.K., Bew J., Simpson P.J., Ross P.R., Stanton C. 2003. Enumeration of probiotic pediococci in animal feed: Interlaboratory study. J. AOAC 86, 791-801
- [5] Technical dossier, section II, 2. Characterisation of the active agent
- [6] Technical dossier, section II, Annexes 'genetic analysis of the MA 18/5M strain'
- [7] Technical dossier, section II, 2.5. Control methods

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was the Community Reference Laboratory for Feed Additives (CRL-FA), Geel, Belgium. This initial evaluation report is made available for commenting to the consortium of National Reference Laboratories.

8. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was the Community Reference Laboratory for Feed Additives (CRL-FA), Geel, Belgium. The initial evaluation report was made available for commenting to the consortium of National Reference Laboratories.

9. ACKNOWLEDGEMENTS

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- NRL – RP Praha, Praha, Czech Republic
- French NRL-FA, Rennes, France
- Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, Oberschleißheim, Germany
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