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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-2008-421 FAD-2008-0015
Name of Additive:	Bactocell PA 10 or Fermaid PA 10
Active Agent(s):	*Pediococcus acidilactici* CNCM MA 18/5M
Rapporteur Laboratory:	Community Reference Laboratory for Feed Additives (CRL-FA)
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EXECUTIVE SUMMARY

In the current application authorisation is sought for Bactocell PA 10 or Fermaid PA 10 under the category 'zootechnical additives', functional group 'gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003. Specifically, the use of Bactocell PA 10 for shrimps is requested. Bactocell concentrate consists of 10^{10} viable cells c.f.u. (colony forming units) per gram of *Pediococcus acidilactici* CNCM MA 18/5M as active agent. The feed additive can be effectively used in any feed for shrimps at a recommended dose of 1 x 10^9 to 1 x 10^{10} 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*, a ring-trial validated method is proposed. 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, respectively [J. AOAC 2003, 86, 791-801]. This method is recommended for official controls to determine colony forming units in the frame of the authorisation. The spread plate method has a limit of quantification (LOQ) of around 10^7 c.f.u./kg which is well below the anticipated concentrations of the active agent in feedingstuffs.

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 [J. Microbiol. Methods 2006, 64, 120-125].

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

KEYWORDS

Bactocell PA 10, Fermaid PA 10, feed additive, shrimps, zootechnical, *Pediococcus acidilactici*

1. BACKGROUND

Bactocell PA 10 or Fermaid PA 10 is a product for which authorisation is sought under the category ‘zootechnical additives’, functional group 'gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003. Bactocell PA 10 contains a minimum of 1 x 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 shrimps, by mixing the feed additive into feedingstuffs at a proposed dose of 1 10^{9} to 1 x 10^{10} 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 10 or Fermaid PA 10 dossier (EFSA-Q-2008-421) 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 product 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 [7].

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), mercury (Hg), lead (Pb), selenium and aflatoxin B_1 using appropriate methodologies [8]. Dioxin and dioxin-like PCBs were determined by an external laboratory [9]. Microbiological quality of the additive was ensured by examination for *Salmonella* species, pathogenic staphylococci, coliforms, *Escherichia coli*, yeasts, anaerobic sulphite reducing bacteria and enterococci by using suitable methods [8]. 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 Bactocell PA 10 or Fermaid PA 10, the applicant proposes a ring trial validated spread plate method using Man Rogosa Sharp (MRS) agar which is appropriate for official controls [4]. In the proposed method is a sample suspended in a dilution buffer and decimally diluted followed by a transfer of appropriate dilutions to agar plates. The agar plates are incubated at 37 °C for 48 - 72 h.

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, respectively [4]. This method is recommended for

official controls. The spread plate method has a limit of quantification (LOQ) of around 10 x 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 [7].

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 [7].

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 Bactocell PA 10 or Fermaid PA 10 for shrimps 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. 2.2. Biological origin
[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, Annex 'Genetic analysis of the MA 18/5M strain'
[7] Simpson P.J., Fitzgerald G.F., Stanton C., Ross R.P. 2006. Enumeration and identification of pediococccci in powder-based products using selective media and rapid PFGE. J. Microbiol. Methods, 64, 120-125
[8] Technical dossier, section II, 2.5. Control methods
[9] Technical dossier, section II, 1.4.1.6. Dioxins

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 was made available for commenting to the consortium of National Reference Laboratories.

9. ACKNOWLEDGEMENTS

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- Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, Oberschleiβheim, Germany
- National Veterinary Research Institute, Pulawy, Poland
- National Veterinary Institute, Ljubljana, Slovenia