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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-2008-337 FAD-2008-0014
Name of Additive:	MLB
Active Agent(s):	*Lactobacillus acidophilus* **DSM** 13241
Rapporteur Laboratory:	Community Reference Laboratory for Feed Additives (CRL-FA)
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EXECUTIVE SUMMARY

In the current application authorisation is sought for the microbial feed additive MLB under the category 'zootechnical additives', functional group 'gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003. Specifically, the use of MLB for dogs and cats is requested. MLB contains a minimum of 1.0×10^{11} viable cells (c.f.u., colony-forming units) of *Lactobacillus acidophilus* DSM 13241 (as active agent) per gram. The feed additive is intended to be mixed into complete feedingstuffs at final concentrations of 6×10^{9} to 1×10^{11} c.f.u./kg for dogs and 3×10^{9} to 2.5×10^{10} c.f.u./kg for cats, respectively.

For the enumeration of the active agent, *Lactobacillus acidophilus* DSM 13241, in the *feed additive*, *premixtures* and *feedingstuffs* a plate count method using MRS agar is proposed by the applicant. For official controls of the active agent *Lactobacillus acidophilus* DSM 13241 in the *feed additive*, *premixtures* and *feedingstuffs* a similar spread plate enumeration method using MRS agar is recommended. This enumeration method was validated in a collaborative study [Food Microbiol. 20 (2003) 57-66]. The method's performance characteristics of the enumeration method are standard deviations for repeatability (s_r) and reproducibility (s_R) of around 0.10 – 0.26 $\log_{10}$ and 0.18 – 0.39 $\log_{10}$ calculated from the base 10 logarithms of the measured c.f.u./g in feedingstuffs, respectively. The limits of quantification (LOQ) of this method are 10^4 colony forming units (c.f.u) per gram (g) feed additive or premixture and 10^7 c.f.u./kg feedingstuff.

The applicant applies pulsed field gel electrophoresis (PFGE) for strain identification of *Lactobacillus acidophilus* DSM 13241. PFGE is a widely recognised methodology for microbial strain identification and is considered suitable for official controls in the frame of the authorisation.

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

KEYWORDS

MLB, zootechnical, *Lactobacillus acidophilus*, dogs, cats

1. BACKGROUND

MLB is a feed additive for which authorisation is sought under the category 'zootechnical additives', functional groups 'gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003. MLB is provided in form of a powder containing at least 1.0 x 10^{11} c.f.u. viable cells of *Lactobacillus acidophilus* DSM 13241 per gram as active agent. The strain is deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) in Braunschweig, Germany [1]. The intended use of the current application are feedingstuffs for dogs and cats, by mixing the feed additive into complete feedingstuffs at final concentrations of 6 x 10^{9} to 1 x 10^{11} c.f.u./kg for dogs and 3 x 10^{9} to 2.5 x 10^{10} c.f.u./kg for cats, respectively [2].

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 MLB dossier (EFSA-Q-2008-377) 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 determination of the quantitative composition of the active agent in the additive is provided by the applicant [3]. The applicant provided validation data for the applicability of the method for the additive [4]. This method is used for enumeration of lactic acid bacteria. The number of viable microorganisms is given in colony forming units (c.f.u.) per g. For official controls a similar validated enumeration method is recommended [5].
The active agent is a strain of *Lactobacillus acidophilus* DSM 13241. A range of techniques are applied by the applicant for identification of *Lactobacillus acidophilus* DSM 13241 comprising microscopy, biochemical and molecular methods [6]. The applicant uses pulsed field gel electrophoresis (PFGE) which is considered as an appropriate method for official controls.

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)

The applicant proposes the same method as above [3] to analyse premixtures and feedingstuffs for *Lactobacillus acidophilus* DSM 13241. The applicant provided validation data for the applicability of the method for feedingstuffs [7]. For official controls the same ring-trial validated method as above is recommended [5]. Performance characteristics of this method obtained in the collaborative study were expressed in terms of standard deviations for repeatability (s_r) and reproducibility (s_R) calculated from the base 10 logarithms of the measured c.f.u./g. The method's performance characteristics of the enumeration method are standard deviations for repeatability (s_r) and reproducibility (s_R) of around 0.10 – 0.26 $\log_{10}$ and 0.18 – 0.39 $\log_{10}$ calculated from the base 10 logarithms of the measured c.f.u./g in feedingstuffs, respectively. The limits of quantification (LOQ) of this method are 10^4 colony forming units (c.f.u) per gram (g) feed additive or premixture and 10^7 c.f.u./kg feedingstuff.
Concerning the unambiguous identification of the specific strain *Lactobacillus acidophilus* DSM 13241 in the feed additive, premixtures and feedingstuff PFGE is used [6]. This methodology is widely accepted and used for microbial identifications and is therefore considered suitable for official controls in the frame of this authorisation.

4. CONCLUSIONS AND RECOMMENDATIONS

The applicant provided methods for the enumeration and identification of the active agent *Lactobacillus acidophilus* DSM 13241 in the feed additive, premixtures and feedingstuffs. A ring-trial validated enumeration method and PFGE are considered appropriate for official controls.

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

Enumeration: Spread plate method using MRS agar and an incubation temperature of 37 ºC
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 MLB for dogs and cats have been sent to the Community Reference Laboratory for Feed Additives. The dossier has been made available to the CRL by EFSA.

6. REFERENCES

[1] Technical dossier. Annexes, A05 DSMZ Safe deposit certificate

[2] Proposal of Register entry Annex III

[3] Technical dossier. Annexes, A23a Enumeration of microorganisms – MRS Agar

[4] Technical dossier. Annexes, 14a Validation of viable cell count method for MLB powder

[5] Leuschner R.G.K., Bew J., Coeuret V., Vernoux J.-P., Gueguen M. 2003. A collaborative study of a method for the enumeration of probiotic lactobacilli in animal feedingstuff. Food Microbiol. 2003. 20, 57-66

[6] Technical dossier. Annexes, A7a,b;8 Genetic stability of *Lactobacillus acidophilus* DSM 13241; DNA fingerprinting

[7] Technical dossier. Annexes, 14b Validation of viable cell count method for dry pet food

7. RAPPORTEUR LABORATORY

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.

8. ACKNOWLEDGEMENTS

The following National Reference Laboratories contributed to this report:

- National Feed Laboratory, Lublin, Poland
- National Veterinary Institute, Ljubljana, Slovenia