



JRC.DDG.D.6/CvH/DM/hn/ARES (2010) 213021

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-2008-0020
CRL/080015

Name of Additive: Biomin IMB 52

Active Agent (s): *Enterococcus faecium* DSM 3530

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Date: 16/04//2010

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Date: 23/04/2010

EXECUTIVE SUMMARY

In the current application authorisation is sought for the microbial feed additive *Enterococcus faecium* DSM 3530 under the category 'zootechnical additives', functional group 4(b), 'gut flora stabilisers' according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorization is sought for the use of Biomin IMB 52 for piglets. The *feed additive* is presented as a white – yellow powder with a concentration of 1×10^{10} CFU/g. It is intended to be mixed at a dose ranging from 5×10^8 to 1×10^9 CFU/kg of complete *feedingstuffs*.

For the enumeration of *Enterococcus faecium* DSM 3530 in *feed additives*, *premixtures* and *feedingstuffs* the CRL recommends CEN spread plate method using Bile Esculin Azide Agar (EN 15788). The performance characteristics of the CEN method reported after logarithmic transformation (CFU) are:

- a repeatability standard deviation (s_r) ranging from 0.12 to 0.2 \log_{10} CFU/g,
- a reproducibility standard deviation (s_R) ranging from 0.23 to 0.41 \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 (5×10^8 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.

Further testing or validation is not considered necessary.

KEYWORDS

Enterococcus faecium DSM 3530, zootechnical additives, piglets, gut flora stabilisers.

1. BACKGROUND

Enterococcus faecium DSM 3530 is a feed additive for which authorisation under Article 4(1) is sought under the category of 'zootechnical additives' functional group 4(b), 'gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003 [1]. The strain is deposited at the 'Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ)' in Braunschweig, Germany [2]. Biomin IMB 52 is a white – yellow powder with a concentration of 1×10^{10} CFU/g of *Enterococcus faecium* DSM 3530. It is intended to be mixed at a dose ranging from 5×10^8 to 1×10^9 CFU/kg of complete *feedingstuffs* for piglets [3].

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 tasks of the Community Reference Laboratory concerning applications for authorizations 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 dossier, the methods of analysis submitted in connection with the Biomin IMB 52 dossier 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 characterisation of the strain *Enterococcus faecium* DSM 3530 the applicant used routine microbiological criteria (microscopy, growth characteristics, biochemical fermentation) as well as electrophoretic DNA- and protein-patterns. These methods are suitable for the purpose of analysis [4].

The CRL recommends for official control, Pulsed Field Gel Electrophoresis (PFGE), a generally recognised standard methodology for genetic identification [5].

Qualitative and quantitative composition of any impurities in the additive

The applicant analysed the *feed additive* for microbial contaminants (such as coliform bacteria, *Escherichia coli*, *Salmonella*, yeasts and moulds) by using appropriate tests [6].

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 *Enterococcus faecium* DSM 3530 in *feed additive, premixtures and feedingstuffs*, the applicant proposes in house validated pour plate method according to Koch [7]. The sample is suspended and diluted in a buffer solution; the appropriate dilutions are then transferred into petri dishes and MRS (de Man, Rogosa, Sharp) agar is added. When the agar is solidified, plates are incubated at 37°C for 48 hours before colony counting. The CRL notes that MRS medium is not specifically designed for cultivation of *Enterococcus* species but for cultivation of 'lactic acid bacteria' such as *Lactobacillus*, *Pediococcus* and *Enterococcus*.

Another internationally recognised spread plate method for enumeration of *Enterococcus* spp (EN 15788) should be used instead [8]. The sample is suspended and diluted, the appropriate dilutions are then spread on Bile Esculin Azide Agar. The agar plates are incubated at 37 °C for 24 hours before colony counting. The performance characteristics of the CEN method reported after logarithmic transformation (CFU) are:

- a repeatability standard deviation (s_r) ranging from 0.12 to 0.2 \log_{10} CFU/g,
- a reproducibility standard deviation (s_R) ranging from 0.23 to 0.41 \log_{10} CFU/g and

- a limit of detection (LOD) of 1×10^5 CFU/kg [9], well below the minimum dose proposed by the applicant (5×10^8 CFU/kg of *feedingstuffs*).

The CRL recommends, for official control, CEN method (EN 15788) for the enumeration of *Enterococcus faecium* DSM 3530 in *feed additives, premixtures and feedingstuffs*.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the CRL recommends the CEN method - EN 15788 - for the enumeration of the active agent *Enterococcus faecium* DSM 3530 in *feed additive, premixtures and feedingstuffs*.

For the analysis of the identity of the bacterial strain, *Enterococcus faecium* DSM 3530 the CRL recommends Pulsed Field Gel Electrophoresis (PFGE) for official control.

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 Bile Esculin Azide agar (EN 15788)
- 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 Biomin IMB 52 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.1831/024-2008
- [2] *Technical dossier, section II/ 2.2. Characterisation of the additive
- [3] *Application, Proposal for Register Entry, Annex A
- [4] *Annex_II_5_4_Differentiation_Foissy
- [5] European Community Project SMT4-CT98-2235.'Methods for the Official Control of Probiotics Used as Feed Additives, Volume 1. 2002. Report 20873-1. Office for official Publications of the European Communities. ISBN 92-894-6250-7 (Vol. I)
- [6] *Annex_II_3_2_Microbiological Methods
- [7] *Annex_II_7_1_SOP_cc_IMB52 encapsulated
- [8] EN 15788 'Animal feeding stuffs - Isolation and enumeration of Enterococcus (*E. faecium*) spp'
- [9] ISO 7218 'Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations'

*Refers to Dossier no: FAD-2008-0020

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

The following National Reference Laboratories contributed to this report:

- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes (FR)

- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Instytut Zootechniki w Krakowie, Krajowe Laboratorium Pasz, Lublin (PL)
- Skúšobné laboratórium – Oddelenie analýzy krmív, Ústredný kontrolný a skúšobný ústav poľnohospodársky, Bratislava (SK)