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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-2010-0066
CRL/ 100079

Name of product: Biomin® MTV

Active Agent (s): *Trichosporon mycotoxinivorans*
DSM 14153

Rapporteur Laboratory: Community Reference Laboratory for
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Date: 06/12/2010

EXECUTIVE SUMMARY

In the current application authorisation is sought under article 4(1) for *Biomin*[®] *MTV* under the category 'zootechnical additives', functional group 4(b) 'gut flora stabilisers', according to Annex I of Regulation (EC) No 1831/2003. Specifically, the authorisation is sought for the use of *Biomin*[®] *MTV* for weaned piglets. The active substance is a pure culture of yeast strain *Trichosporon mycotoxinivorans* DSM 14153, with a minimum concentration of 5×10^7 CFU/g. It is intended to be mixed to complete *feedingstuffs* at a dose ranging from 5×10^7 to 1×10^8 CFU/kg.

For the enumeration of *Trichosporon mycotoxinivorans* DSM 14153 in *feed additive*, *premixtures* and *feedingstuffs* the Applicant proposes a single laboratory validated Koch's pour plate method. However, the CRL recommends instead, the ring trial validated CEN pour plate method for the enumeration of yeast probiotic strains (EN 15789), using yeast extract dextrose chloramphenicol agar (CGYE). The performance characteristics of the EN 15789 method reported after logarithmic transformation (CFU) are:

- a repeatability standard deviation (s_r) ranging from 0.17 to 0.36 \log_{10} CFU/g,
- a reproducibility standard deviation (s_R) ranging from 0.55 to 0.60 \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 (5×10^7 CFU/kg of *feedingstuffs*).

Molecular methods were used by the Applicant for identification of the active agent. The CRL recommends for official control Polymerase Chain Reaction (PCR), a generally recognised standard methodology for identification of yeasts.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.

KEYWORDS

Trichosporon mycotoxinivorans DSM 14153, zootechnical additives, weaned piglets, gut flora stabilisers.

1. BACKGROUND

In the current application authorisation is sought under article 4(1) for *Biomin[®] MTV* under the category 'zootechnical additives', functional group 4(b) 'gut flora stabilisers', according to Annex I of Regulation (EC) No 1831/2003 [1]. The active component of *Biomin[®] MTV* is a pure culture of the strain *Trichosporon mycotoxinivorans* DSM 14153, with a minimum concentration of 5×10^7 CFU/g [2]. The strain is deposited in the 'Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ)' in Braunschweig, Germany [3]. Specifically, the authorisation is sought for the use of *Biomin[®] MTV* for weaned piglets. It is intended to be mixed to complete *feedingstuffs* at a dose ranging from 5×10^7 to 1×10^8 CFU/kg of *Trichosporon mycotoxinivorans* DSM 14153 [4].

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 the 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 or group of applications. For this dossier, the methods of analysis submitted in connection with *Biomin[®] MTV*, 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 impurities in the additive

For identification and characterisation of the strain *Trichosporon mycotoxinivorans* DSM 14153 the Applicant used molecular methods such as 18S rDNA sequence analysis and Amplified Fragment Length Polymorphism (AFLP) [5,6]. These methods are suitable for the purpose of analysis. However, the CRL recommends for official control Polymerase Chain Reaction (PCR), a generally recognised standard methodology for identification of yeasts [7].

Qualitative and quantitative composition of any impurities in the additive

The Applicant analysed the *feed additive* for microbial contaminants (such as *Escherichia coli*, *Salmonella*, coliforms and moulds) by using appropriate ISO tests [8,9].

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 the active substance in feed additive, premixtures, feedingstuffs and water

For the enumeration of *Trichosporon mycotoxinivorans* DSM 14153 in *feed additive*, *premixtures* and *feedingstuffs* the Applicant proposes a single laboratory validated Koch's pour plate method [10]. The sample is suspended and diluted in a saline solution with added Tween 80; the appropriate dilutions are then transferred into Petri dishes and wort agar is added. When the agar is solidified, plates are incubated at 37°C for 24 to 48 hours before colony counting.

A ring trial validated pour plate method for the enumeration of yeast probiotic strains (EN 15789) was developed by CEN, using yeast extract dextrose chloramphenicol agar (CGYE) [11]. The sample is suspended in phosphate buffered saline (PBS) and diluted in a peptone salt solution. The appropriate dilutions are then transferred to Petri dishes and melted CGYE agar is added. When the agar is solidified, plates are incubated at 35°C for 48 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 0.17 to 0.36 \log_{10} CFU/g,
- a reproducibility standard deviation (s_R) ranging from 0.55 to 0.60 \log_{10} CFU/g; and
- a limit of detection (LOD) of 1×10^5 CFU/kg [12], well below the minimum dose proposed by the applicant (5×10^7 CFU/kg of *feedingstuffs*).

Based on these performance characteristics the CRL recommends, for official control, the CEN method EN 15789 for the enumeration of *Trichosporon mycotoxinivorans* DSM 14153 in *feed additives*, *premixtures* and *feedingstuffs*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the CRL recommends the CEN method - EN 15789 - for the enumeration of the active agent *Trichosporon mycotoxinivorans* DSM 14153 in *feed additive, premixtures* and *feedingstuffs*.

For the identification of the yeast strain *Trichosporon mycotoxinivorans* DSM 14153 the CRL recommends Polymerase Chain Reaction (PCR) for official control.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories in accordance with article 10 of Commission Regulation (EC) No 378/2005 is not considered necessary.

Recommended text for the register entry (analytical method)

- Enumeration: Pour plate method using yeast extract dextrose chloramphenicol (CGYE) agar – CEN method EN 15789
- Identification: Polymerase Chain Reaction (PCR)

5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Biomim MTV* 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] *Application/Ref:SANCO/D/2:Forw.Appl.1831/0046-2010.
- [2] *Technical dossier, Section II/2.1.3. Qualitative and quantitative composition
- [3] *Technical dossier, Section II, Annex_II_03
- [4] *Application, Proposal for Register Entry, Annex A
- [5] *Technical dossier, Section II, Annex_II_01
- [6] *Technical dossier, Section II, Annex_II_14
- [7] 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)
- [8] *Technical dossier, Section II, Annex_II_06
- [9] *Technical dossier, Section II, Annex_II_07
- [10] *Technical dossier, Section II, Annex_II_32
- [11] EN 15789:2009 'Animal feeding stuffs - Isolation and enumeration of yeast probiotic strains'
- [12] ISO 7218:2007 'Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations'

*Refers to Dossier no: FAD-2010-0066

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

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, as last amended by Regulation (EC) No 885/2009.

8. ACKNOWLEDGEMENTS

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