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Evaluation Report on the Analytical Methods submitted in connection with the Application for Authorisation of a Feed Additive according to Regulation (EC) No 1831/2003

Biomin BBSH 797
(FAD-2012-0024 ; CRL/120015)

European Union Reference Laboratory

Evaluation Report on the Analytical Methods submitted in connection with the Application for Authorisation of a Feed Additive according to Regulation (EC) No 1831/2003

Dossier related to:	**FAD-2012-0024** **CRL/120015**
Product Name:	**Biomin BBSH 797**
Active Substance(s):	**Micro-organism DSM 11798, *Genus novus* of the *Coriobacteriaceae* family**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **Geel, Belgium**
Report prepared by:	**Dijana Mitić (EURL-FA)**
Report checked by:	**Piotr Robouch (EURL-FA)**
Date:	**13/02/2013**
Report approved by:	**Christoph von Holst**
Date:	**13/02/2013**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4(1) for BIOMIN BBSH 797, under the category/functional group 1(m), "technological additives/ substances for reduction of the contamination of feed by mycotoxins", according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the *feed additive* for all animal species and categories. The active agent of BIOMIN BBSH 797 is the micro-organism DSM 11798; temporarily defined as *Genus novus species novus* DSM 11798 of the *Coriobacteriaceae* family. BIOMIN BBSH 797 is intended to be used as a *feed additive* based on its specific mycotoxin - deoxynivalenol (DON) -biotransformation activity.

The *feed additive* is to be placed on the market as a powder, containing a minimum concentration of $1x10^{10}$ colony forming units (CFU)/g of *Genus novus species novus* DSM 11798. It is intended to be incorporated into *feedingstuffs* via *premixtures,* with a final concentration ranging from $1.7x10^{8}$ to $2.2x10^{9}$ CFU/kg *feedingstuffs*.

For the enumeration of *Genus novus species novus* DSM 11798 in *feed additive, premixtures* and *feedingstuffs* the Applicant proposed a single-laboratory validated and further verified pour plate method, using VM agar supplemented with Oxyrase. The performance characteristics of the method reported after logarithmic transformation in *feed additive*, *premixtures* and *feedingstuffs* are:

- a standard deviation for *precision* ranging from 0.02 to 0.07 $\log_{10}$ CFU/g;
- a recovery rate (R_{Rec}) ranging from 99 to 103 %; and
- a limit of detection (LOD) of 10^{5} CFU/kg *feedingstuffs.*

Based on the performances characteristics presented, the EURL recommends for official control the single-laboratory validated and further verified pour plate method for the determination of *Genus novus species novus* DSM 11798 in the *feed additive, premixtures* and *feedingstuffs*.

Molecular methods were used by the Applicant to identify the active agent in the *feed additive*. The EURL recommends instead for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised standard methodology for microbial identification.

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

Genus novus species novus DSM 11798, *Coriobacteriaceae*, technological additives, substances for reduction of the contamination of feed by mycotoxins, all animal species and categories.

1. BACKGROUND

In the current application authorisation is sought under Article 4(1) (new authorisation) for BIOMIN BBSH 797, under the category/functional group 1(m), "technological additives/ substances for reduction of the contamination of feed by mycotoxins", according to the classification system of Annex I of Regulation (EC) No 1831/2003 [1]. Specifically, authorisation is sought for the use of the *feed additive* for all animal species and categories. BIOMIN BBSH 797 is intended to be used as a *feed additive* based on its specific deoxynivalenol (DON)-biotransformation activity. The strain detoxifies this mycotoxin by selective cleavage of its toxic 12,13 – epoxy group, resulting in the harmless metabolite DOM-1 [2].

The active agent of BIOMIN BBSH 797 is the micro-organism DSM 11798; temporarily defined as *Genus novus species novus* DSM 11798 of the *Coriobacteriaceae* family [3,4]. This strain was formerly known as *Eubacterium* [5] and the full taxonomic classification is currently pending. The strain is deposited in the 'Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ)' in Braunschweig, Germany [6].

The *feed additive* is to be placed on the market as a powder, containing a minimum concentration of $1x10^{10}$ CFU/g of *Genus novus species novus* DSM 11798 [4]. It is intended to be incorporated into *feedingstuffs* via *premixtures,* with a final concentration ranging from $1.7x10^{8}$ to $2.2x10^{9}$ CFU/kg *feedingstuffs* [3, 7].

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 European Union Reference Laboratory concerning applications for authorisations of feed additives, as last amended by Regulation (EC) No 885/2009, the EURL is requested to submit a full evaluation report to the European Food Safety Authority (EFSA) for each application, or for each group of applications. For this particular dossier, the methods of analysis submitted in connection with the BIOMIN BBSH

797 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 *Genus novus species novus* DSM 11798 strain the Applicant used morphological, physiological and molecular methods, such as 16S rDNA sequence analysis [8]. These methods are suitable for the purpose of analysis. However, the EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised standard methodology for microbial identification [9].

Qualitative and quantitative composition of impurities in the additive

The Applicant analysed the *feed additive* for microbial contaminants (such as coliforms, *Escherichia coli*, Salmonella spp., yeasts and moulds) by using appropriate EN ISO and AOAC tests [10]. For undesirable substances (i.e. arsenic, cadmium, mercury, lead, selenium, copper, zinc, chrome, aflatoxins) internationally recognised standard methods are available at the respective European Union Reference Laboratory, in accordance with Commission Regulation (EC) No 776/2006.

Description of the analytical methods for the determination of active substance in feed additive and silage

For the enumeration of *Genus novus species novus* DSM 11798 in *feed additive, premixtures* and *feedingstuffs* the Applicant proposed a single-laboratory validated [10,11] and further verified [12-14] pour plate method, based on Koch's protocol. The method was further optimised for the enumeration of the *Genus novus species novus* DSM 11798, using a special medium (VM agar) under anaerobic conditions and low redox potential. The VM agar - free of any sugar sources - consists of agar-agar, peptine, L-arginine, yeast extract, with mineral constituents (such as potassium phosphate and sulphate, magnesium sulphate and calcium chloride) and a vitamin mixture (including pyroxidine, thiamine, riboflavin, nicotinamide and pantothenate) [15]. The samples (1 g for *feed additive* and *premixtures* and 10 g for *feedingstuffs*) are mixed and diluted in an anaerobic buffer solution, supplemented with 1% Cysteine-Na_2S and 2.5% phosphate buffer; and degassed with H_2 flow. The appropriate dilutions are then transferred into Petri dishes and mixed with VM agar supplemented with Oxyrase (a sterile enzyme additive, creating low oxygen conditions). The agar plates are

incubated anaerobically at 37 °C for a minimum of 5 days. The Applicant considers very unlikely interferences from other anaerobic genus present in the feed, except when analyzing complete feedingstuffs containing silages with a considerably high contamination with *Clostridium* species (provided that these species can grow on sugar-free media).

The working range of the method is from 10^2 to 10^{12} CFU/g and the performance characteristics (reported after logarithmic transformation) are presented in Table 1. Furthermore, a limit of detection (LOD) of the method is set to 10^5 CFU/kg *feedingstuffs* [16].

Based on the performances characteristics presented, the EURL recommends for official control the single-laboratory validated and further verified pour plate method for the determination of *Genus novus species novus* DSM 11798 in the *feed additive*, *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.

Table 1: Performance characteristics for the enumeration of *Genus novus species novus* DSM 11798 in the *feed additive (FA), premixtures (PM)* and *feedingstuffs (FS)*

		s_r (lg_{10}(CFU/g))		s_{ip} (lg_{10}(CFU/g))		R_{Rec} (%)	
	lg_{10}(CFU/g)	Validation	Verification	Validation	Verification	Validation	Verification
FA [12]	10.37	0.07	0.02	-	0.02	101	100
PM [13]	8.37	0.07	0.04	-	0.04	101	99
FS [14]	6.26	0.04	0.03	-	0.03	103	99

s_r,and s_{ip}: standard deviation for *repeatability* and *intermediate precision*, respectively.

R_{Rec}: a recovery rate

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control the single-laboratory validated and further verified pour plate method for the enumeration of *Genus novus species novus* DSM 11798 in the *feed additive, premixtures* and *feedingstuffs* and Pulsed Field Gel Electrophoresis (PFGE) for its identification.

Recommended text for the register entry (analytical method)

- Enumeration of *Genus novus species novus* DSM 11798 in the *feed additive, premixtures* and *feedingstuffs*: pour plate method using VM agar supplemented with Oxyrase
- Identification of *Genus novus species novus* DSM 11798: Pulsed Field Gel Electrophoresis (PFGE)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, samples of the additive BIOMIN BBSH 797 have been sent to the European Union Reference Laboratory for Feed Additives. The dossier has been made available to the EURL by EFSA.

6. REFERENCES

[1] *Application/Ref: SANCO/G1: Forw.Appl.1831/0042-2012
[2] *Technical Dossier, Section II.2.1 Identity of the additive
[3] *Application, Annex A, Proposal for register entry
[4] *Technical Dossier, Section II, Annex_II_02_annex entry
[5] *Technical Dossier, Section II, Annex_II_01_genus and species_historic background
[6] *Technical Dossier, Section II, Annex_II_03_strain deposit certificate
[7] *Technical Dossier, Section II, 2.5 Conditions of use
[8] *Technical Dossier, Section II, 2.2 Characterisation of the active substance/agent
[9] European Community Project SMT4-CT98-2235.*"Methods for the Official Control of Probiotics Used as Feed Additives,* Report 20873/1 EN (2002) ISBN 92-894-6250-7 (Vol. I)"
[10] *Technical Dossier, Section II, Annex_II_59_SOP colony count
[11] *Technical Dossier, Section II, Annex_II_68_Validation report

[12] *Technical Dossier, Section II, Annex_II_70_CRL form verification_1_pure additive
[13] *Technical Dossier, Section II, Annex_II_71_CRL form verification_2_premix
[14] *Technical Dossier, Section II, Annex_II_72_CRL form verification_3_feed
[15] *Technical Dossier, Section II, Annex_II_59_SOP colony count
[16] ISO 7218:1996 - Microbiology of food and animal feedingstuffs – General rules for microbiological examinations

*Refers to Dossier No: FAD-2012-0024

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was European Union 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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