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

Dossier related to:	FAD-2009-0037 CRL/090019
Product Name:	Prostora Max
Active Substance(s):	*Bifidobacterium animalis* AHC7
Rapporteur Laboratory:	Community Reference Laboratory for Feed Additives (CRL-FA)
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Date:	21/05/2010
Report approved by:	Christoph von Holst
Date:	07/06/2010

EXECUTIVE SUMMARY

In the current application authorisation is sought for the microbial feed additive *Bifidobacterium animalis* AHC7 under the category 'zootechnical additives', functional group 'gut flora stabiliser' according to Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the feed additive placed on the market in the form of pills containing *Bifidobacterium animalis* AHC7. It is intended to be used, to guarantee a minimum daily dosage of $1x10^9$ CFU *Bifidobacterium animalis* AHC7. The product is marketed in a sealed tray, containing 15 individually pills, for a total of one week supplementation. There are no target levels of the feed additive in complete feedingstuffs.

For the enumeration of *Bifidobacterium animalis AHC7* strain in *feed additives* the applicant proposed the method EN 15785. The performance characteristics of the ring trial validated method reported after logarithmic transformation (CFU) are:

- a repeatability standard deviation (s_r) ranging from 0.13 to 0.15 $\log_{10}$ CFU/g,
- a reproducibility standard deviation (s_R) ranging from 0.24 to 0.25 $\log_{10}$ CFU/g, and
- a limit of detection (LOD) of $1x10^5$ CFU/kg in *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

Bifidobacterium animalis AHC7, zootechnical additives, dogs, gut flora stabilisers.

1. BACKGROUND

Prostora Max is a feed additive for which authorisation under Article 4(1) is sought under the category of 'zootechnical additives' functional group 'gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003 [1]. Specifically, authorisation is sought for the feed additive placed on the market in the form of pills containing *Bifidobacterium animalis* AHC7 [2]. The strain of *Bifidobacterium animalis* AHC7 was deposited with the National Collection of Industrial, Marine and Food Bacteria (NCIMB) with accession number 41617 [3]. *Prostora Max* is intended to be used to guarantee a minimum daily dosage of $1x10^9$ CFU *Bifidobacterium animalis* AHC7 [4]. The product is marketed in a sealed tray, containing 15 individually pills, for a total of one week supplementation [4].

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 authorizations of *feed additives*, as last amended by Regulation (EC) No 885/2009, the CRL 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 *Prostora Max* 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 characterization of the strain *Bifidobacterium animalis* AHC7 the applicant used Rep-PCR (Repetitive element sequence-based PCR) for strain typing. This method is suitable for the purpose of analysis [5]. However, the CRL recommends for official controls pulsed field gel electrophoresis (PFGE), a generally recognised standard methodology for microbial identification [6].

Qualitative and quantitative composition of any impurities in the additive

The applicant analysed the *feed additive* for microbial contaminants (such as Enterobacteria, Escherichia coli, Salmonella and yeasts) by using appropriate EN ISO tests [7]. 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

The product is placed in the market as "ready to use" pills. For enumeration of *Bifidobacterium animalis* AHC7 in *feed additive,* the applicant proposes the ring trial validated CEN method - EN 15785 - an internationally recognised spread plate method. The sample is suspended and diluted in a buffer solution; the appropriated dilutions are then spread on MRS (de Man, Rogosa, Sharp) agar plates. The agar plates are incubated at 37°C for 48 hours [8]. The performance characteristics of the CEN method reported after logarithmic transformation (CFU) are:

- a repeatability standard deviation (s_r) ranging from 0.13 to 0.15 $\log_{10}$ CFU/g,
- a reproducibility standard deviation (s_R) ranging from 0.24 to 0.25 $\log_{10}$ CFU/g, and
- a limit of detection (LOD) of $1x10^5$ CFU/kg in *feedingstuffs* [9].

Based on these acceptable performance characteristics, the CRL recommends, for official controls the CEN method (EN 15785:2009) for the enumeration of *Bifidobacterium animalis* AHC7 in *feed additives*.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the CRL recommends the CEN method - EN 15785:2009 - for the enumeration of the active agent *Bifidobacterium animalis* AHC7 in the *feed additive*.

For the analysis of the identity of the bacterial strain, *Bifidobacterium animalis* AHC7 the CRL recommends Pulsed Field Gel Electrophoresis (PFGE) for official control [5].

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

- Enumeration: Spread plate method using MRS agar (EN 15785:2009)
- 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 *Bifidobacterium animalis* AHC7 for dogs 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.1831/032-2009

[2] *Application, Annex A, Proposal for register entry

[3] *Technical Dossier, Section II 2.2.1.3 Micro-organisms, "Certificate of deposition"

[4] *Technical Dossier, Section II 2.5.1 Proposed mode of use

[5] *Technical Dossier, Annex_II_15_AgentGenStab.pdf

[6] 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)

[7] *Technical dossier 2.3.1.2. Certification, "Annex_II_19_AgentValid.pdf"

[8] EN 15785:2009 *"Animal feeding stuffs- Isolation and enumeration of Bifidobacterium spp."*

[9] ISO 7218:2007 *"Microbiology of food and animal feeding stuffs- General requirements and guidance for microbiological examinations"*

*Refers to Dossier no: FAD-2009-0037

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

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- Instytut Zootechniki w Krakowie, Krajowe Laboratorium Pasz, Lublin (PL)
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