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Community Reference Laboratory for Feed Additives

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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-2008-302 FAD-2008-0006
Name of Additive:	Biosprint®
Active Agent(s):	*Saccharomyces cerevisiae* BCCM/MUCL 39885
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 Biosprint® under the category 'zootechnical additives', functional group 'gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003. Specifically, the use of Biosprint® for sows is requested. Biosprint® in one of its three commercialised forms: powder, spherical or oval granulated contains a minimum of 1 x 10^9 viable cells (c.f.u., colony-forming units) of *Saccharomyces cerevisiae* BCCM/MUCL 39885 (as active agent) per gram. The feed additive is intended to be mixed into complete feedingstuffs at a final concentration of 6.4 x 10^9 to 1.9 x 10^{10} c.f.u./kg.

For the determination of the active agent, *Saccharomyces cerevisiae* BCCM/MUCL 39885, in the *feed additive*, a pour plate method based on ISO 7954 and a molecular identification method (polymerase chain reaction (PCR)) are proposed by the applicant, which are considered appropriate. For the determination of the active agent *S. cerevisiae* BCCM/MUCL 39885 in *premixtures* and *feedingstuffs* the same methods are proposed by the applicant. The enumeration method was validated in a collaborative study [System. Appl. Microbiol. 2003. 26, 147-153]. The performance characteristics of the enumeration method are standard deviations for repeatability (s_r) and reproducibility (s_R) of around 0.17 – 0.36 $\log_{10}$ and 0.55 – 0.60 $\log_{10}$ 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 around 1000 colony forming units (c.f.u) per gram (g) feed additive or premixture and around 10^6 c.f.u./kg feedingstuff. These performance characteristics are considered acceptable. This method is recommended for official controls of the active agent expressed in c.f.u. in the feed additive, premixtures and feedingstuffs.

The PCR method for strain identification was ring trial validated and demonstrated a high level of correct identification between laboratories [System. Appl. Microbiol. 2004. 27, 492-500]. It is therefore considered appropriate for official controls.

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

KEYWORDS

Biosprint®, yeast, zootechnical, *Saccharomyces cerevisiae*, sows

1. BACKGROUND

Biosprint® is a feed additive for which authorisation is sought under the category 'zootechnical additives', functional group 'gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003. Biosprint® contains at least 1 x 10^9 c.f.u. viable cells of *Saccharomyces cerevisiae* BCCM/MUCL 39885 per gram as active agent. The strain is deposited at the Belgian Coordinated Collection of Microorganisms and registered as *Saccharomyces cerevisiae* BCCM/MUCL 39885 [1]. The intended use of the current application are feedingstuffs for sows, by mixing the feed additive into complete feedingstuffs at a final concentration of 6.4 x 10^9 to 1.9 x 10^{10} c.f.u./kg [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 Biosprint® dossier (EFSA-Q-2008-302) 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. The number of viable microorganisms is given in colony forming units (c.f.u.) per g and was provided by the applicant. The proposed method

was fully ring-trial validated and is recommended for official controls in the frame of the authorisation [3, 4].

The active agent is a strain of the yeast *Saccharomyces cerevisiae* (BCCM/MUCL 39885). For identification of the strain *Saccharomyces cerevisiae* BCCM/MUCL 39885 a published ring trial validated polymerase chain reaction (PCR) method is recommended [5, 6]. This method is considered appropriate 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 same method as above is proposed to analyse premixtures and feedingstuffs for Biosprint® [3, 4]. The validated pour plate method is based on ISO 7954 and uses chloramphenicol glucose yeast extract (CGYE) agar. In the proposed method is a sample suspended in a dilution buffer and decimally diluted followed by a transfer of appropriate dilutions to agar plates. Incubation of CGYE plates was carried out at 35 ºC for two days. 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). Analysis results of samples of feedingstuffs with a mean concentration of 7.13 $\log_{10}$ c.f.u./g had a repeatability standard deviation s_r of 0.17 $\log_{10}$ and a reproducibility standard deviation s_R of 0.55 $\log_{10}$ calculated from the base 10 logarithms of the measured c.f.u./g, respectively. Feedingstuff samples with a concentration of 7.48 $\log_{10}$ c.f.u./g revealed a s_r of 0.36 $\log_{10}$ and a s_R of 0.60 $\log_{10}$ calculated from the base 10 logarithms of the measured c.f.u./g, respectively. The limits of quantification (LOQ) of this method are around 1000 colony forming units (c.f.u) per gram (g) additive or premixtures and around 10^6 c.f.u./kg for feedingstuffs. The method is recommended for official controls.

Concerning the unambiguous identification of the specific strain *S. cerevisiae* BCCM/MUCL 39885 in Biosprint® and in premixtures and feedingstuff supplemented with Biosprint®, the same polymerase chain reaction (PCR) method as above is recommended [5]. This method was validated in a collaborative study which demonstrated a high level of correct identification between laboratories, and it is therefore considered suitable for official controls [6].

4. CONCLUSIONS AND RECOMMENDATIONS

Methods for the enumeration and identification of the active agent *Saccharomyces cerevisiae* BCCM/MUCL 39885 in the feed additive, premixtures and feedingstuffs are proposed for official controls. The methods, both of which are ring-trial validated, comprise a quantification of colony forming units and a strain identification by PCR of the active agent.

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

Enumeration: Pour plate method using chloramphenicol glucose yeast extract agar

Identification: Polymerase chain reaction (PCR) method

5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

In accordance with the requirements of Regulation (EC) No 1831/2003, samples of the additive Biosprint® for sows 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. Section II, Annex 1

[2] Proposal of Register entry Annex III

[3] Technical dossier. Section II, 2.6. Methods of analysis

[4] Leuschner R.G.K., Bew J., Bertin, G. 2003. Validation of an official control method for enumeration of authorised probiotic yeast in animal feedingstuff. System. Appl. Microbiol., 26, 147-153

[5] Nes, F., Lavallée F., Dubourdieu D., Aigle M., Dulau L. 1993. Identification of yeast strains using the polymerase chain reaction. J. Sci. Food Agric., 62, 89-94

[6] Leuschner R.G.K., Bew J., Fourcassier P., Bertin G. 2004. Validation of the Official Control Methods based on polymerase chain reaction (PCR) for identification of authorised probiotic yeast in animal feedingstuff. System. Appl. Microbiol. 27, 492-500

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

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