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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-2007-104 FAD-2007-0013
Name of Additive:	Biosaf® Sc47 for pigs for fattening
Active Agent(s):	*Saccharomyces cerevisiae* NCYC Sc47
Rapporteur Laboratory:	Community Reference Laboratory for Feed Additives (CRL-FA)
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Date:	01/10/2007

EXECUTIVE SUMMARY

In the current application authorisation is sought for the microbial feed additive BIOSAF® Sc47 under the category 'zootechnical additives', functional group 'gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003. Specifically, the use of BIOSAF® Sc47 for pigs for fattening is requested. BIOSAF® Sc47 contains a minimum of 5 x 10^9 of viable cells (c.f.u., colony-forming units) of *Saccharomyces cerevisiae* NCYC Sc47 (as active agent) per gram. The feed additive is intended to be mixed into complete feedingstuffs at a final concentration of 1.25 x 10^9 to 1.00 x 10^{10} c.f.u./kg.

For the determination of the active agent, a strain of *Saccharomyces cerevisiae* NCYC Sc47, in the *feed additive,* a pour plate method 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* NCYC Sc47 in *feedingstuffs* a similar pour plate method for enumeration and the same molecular PCR method for identification of the strain are proposed. The enumeration method was validated in a collaborative study [System. Appl. Microbiol. 2003. 26, 147-153]. The method's 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 100 colony forming units (c.f.u) per gram (g) feed additive or premixture and 10^7 c.f.u./kg feedingstuff to take account of natural background flora. These performance characteristics are considered acceptable. This method is recommended for official controls of the active agent 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

BIOSAF® Sc47, yeast, zootechnical, *Saccharomyces cerevisiae,* pigs

1. BACKGROUND

BIOSAF® Sc47 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. BIOSAF® Sc47 is provided in form of a powder containing at least 5 x 10^9 c.f.u. viable cells of *Saccharomyces cerevisiae* NCYC Sc47 per gram as active agent. The strain is deposited at the National Collection of Yeast Cultures (NCYC) in Norwich, UK. The intended use of the current application is pigs for fattening, by mixing the feed additive into complete feedingstuffs at a final concentration of 1.25 x 10^9 to 1.00 x 10^{10} c.f.u./kg [1, 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 BIOSAF® Sc47 dossier (EFSA-Q-2007-104) 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 methods for determination of the quantitative composition of the active agent in the additive are provided by the applicant. The applicant uses a pour plate method for enumeration of the active agent in the additive which is based on ISO 7954 [3]. 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 is suitable for the intended purpose. However, another but similar and fully ring-trial validated method is recommended for official controls in the frame of the authorisation which is available in the literature[4].

The active agent is a strain of the yeast *Saccharomyces cerevisiae* (NCYC Sc47). For identification of the authorised strain of *Saccharomyces cerevisiae* NCYC Sc47 a published polymerase chain reaction (PCR) method was used [5] which was validated by a collaborative study [6]. This method is considered appropriate for official controls.

Qualitative and quantitative composition of any impurities in the additive

The applicant analyses the feed additive for microbial contaminants such as *Escherichia coli*, *Salmonella* species, *Staphylococcus aureus*, *Clostridium perfringens* spores, mesophilic flora, and total coliforms by using appropriate methods. Heavy metals including lead, arsenic, mercury, cadmium and selenium are analysed using atomic absorption spectroscopy [7]. The methods are considered suitable for the proposed application. Internationally recognised standardised methods such as ISO/CEN standards where available are recommended for official controls in line with current EU legislation.

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)

To analyse feedingstuffs for BIOSAF® Sc47, the applicant proposes a method that was published and validated by a full collaborative study [4]. The validated pour plate method is based on ISO 7954 and uses chloramphenicol glucose yeast extract agar. 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 100 colony forming units (c.f.u) per gram (g) additive or premixtures and 10^7 c.f.u./kg for feedingstuffs to take account of potentially occurring background flora. The method is recommended for official controls.

Concerning the unambiguous identification of the specific strain of *S. cerevisiae* NCYC Sc47 in BIOSAF® Sc47, in the additive, and if necessary in the feedingstuff, a polymerase chain reaction (PCR) method is used [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

The applicant provided methods for the enumeration and identification of the active agent *Saccaromyces cerevisiae* NCYC Sc47 in the feed additive and feedingstuffs. The proposed methods are described in the dossier for BIOSAF® Sc47 and are considered appropriate. For official controls ring-trial validated methods are recommended for the enumeration and identification of *S. cerevisiae* NCYC Sc47 in the feed additive, premixtures and feedingstuffs.

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 BIOSAF® Sc47 for pigs for fattening 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] Proposal of Register entry Annex III

[2] Technical dossier. Section II, Identity of the additive

[3] ISO 7954. 1987. General guidance for enumeration of yeasts and moulds – Colony count technique at 25 ºC

[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] Technical dossier. Section V, Monograph

7. RAPPORTEUR LABORATORY

The Rapporteur Laboratory for this evaluation was the Community Reference Laboratory for Feed Additives (CRL-FA), Geel, Belgium

8. ACKNOWLEDGEMENTS

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- Laboratoire de Rennes, Rennes, France
- National Veterinary Research Institute, Pulawy, Poland
- National Veterinary Institute, Ljubljana, Slovenia