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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-2006-003
Name of Additive:	BIOSAF® Sc47 (dairy small ruminants)
Active Agent(s):	*Saccharomyces cerevisiae* NCYC Sc47
Rapporteur Laboratory:	Community Reference Laboratory for Feed Additive Authorisation, IRMM, Geel, Belgium (CRL-FAA)
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Date:	19/05/2006

EXECUTIVE SUMMARY

In the current application authorisation is sought for BIOSAF® Sc47 under the category 'zootechnical additives', functional group 'other zootechnical additives', according to Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought to use BIOSAF® Sc47 for dairy small ruminants. BIOSAF® Sc47 contains as active agent *Saccharomyces cerevisiae* NCYC Sc47 at a minimum concentration of 5×10^9 colony forming units (c.f.u.) per gram of additive. It is proposed for use in complete feedingstuffs for dairy small ruminants at a concentration between 7×10^8 and 7.5×10^9 c.f.u./kg.

For quantifying the active agent *Saccharomyces cerevisiae* (NCYC Sc47) in the *additive* the ISO 7954 method is provided by the applicant. For official control purposes a similar pour plate method which was validated by an interlaboratory study is recommended [System. Appl. Microbiol. 2003, 26, 147-153].

This same method [System. Appl. Microbiol. 2003, 26, 147-153] is used by the applicant for enumeration of the active agent in *premixtures* and *feedingstuffs*. The method's performance characteristics obtained in the validation study included relative standard deviations for within-laboratory repeatability (RSD_r) and for between-laboratory reproducibility (RSD_R) of around 5 % and 8 %, respectively. The limit of quantification (LOQ) of the method is 10^5 c.f.u./kg sample, corresponding to 100 c.f.u./g, which is well below anticipated concentrations in feedingstuffs. The method is recommended for official control purposes.

For identification of the yeast strain a molecular pulsed field gel electrophoresis (PFGE) and a polymerase chain reaction (PCR) method are proposed by the applicant. For official control purposes the PCR method which performed appropriately in an interlaboratory study is recommended [System. Appl. Microbiol. 2004, 27, 492-500].

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

KEYWORDS

BIOSAF® Sc47, yeast, zootechnical, *Saccharomyces cerevisiae*, dairy small ruminants

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1. BACKGROUND

BIOSAF® Sc47 is a feed additive for which authorisation is sought under the 'zootechnical additives', functional group 'other zootechnical additives', according to Annex 1 of Regulation (EC) No 1831/2003 [1]. It contains viable cells of the yeast strain *Saccharomyces cerevisiae* NCYC Sc47 as the active agent. The yeast strain is deposited at the National Collection of Yeast Cultures (NCYC), Norwich, UK [2].

The intended use (*cf.* EFSA-Q-2006-003) of the current application is for dairy small ruminants, by mixing the feed additive into complete feedingstuffs at concentrations between 7.0×10^8 and 7.5×10^9 colony forming units (c.f.u.) per kg feedingstuff [1].

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 for each application. For this

particular dossier, the suitability of the control methods and validation studies submitted in connection with, *cf*. EFSA-Q-2006-003, was evaluated.

3. EVALUATION

The numbering system under this point refers to that of the Guidelines for the assessment of additives in feedingtuffs, PART II: Enzymes and Micro-organisms. The method protocols and corresponding validation data – if applicable – are given in Section II of the dossier.

Description of some of the methods listed under item 2.5.1 of the Guidelines.

Qualitative and quantitative composition of the additive BIOSAF® Sc47

The ISO 7954 method for quantifying the active agent *Saccharomyces cerevisiae* (NCYC Sc47) in the additive is provided by the applicant [3]. The method is considered appropriate for the purpose. For official control a similar pour plate method which was validated by an interlaboratory study is recommended [4].

For identification of the authorised strain *Saccharomyces cerevisiae* NCYC Sc47 a polymerase chain reaction (PCR) and/or a pulsed field gel electrophoresis (PFGE) method was used by the applicant. Both methods are considered appropriate for the purpose. For official control purposes the polymerase chain reaction (PCR) method which was validated by an interlaboratory study is recommended [5, 6].

Description of the qualitative and quantitative methods for routine control of the active agent in premixtures and feedingstuffs listed under item 2.5.2 of the Guidelines.

To analyse premixtures and feedingstuffs for BIOSAF® Sc47, the applicant proposes a pour plate method that was validated by a full collaborative study [4]. The method has appropriate performance characteristics which were expressed in terms of relative standard deviations for within-laboratory repeatability (RSD_r) and between-laboratory reproducibility (RSD_R) of around 5 % and 8 %, respectively. The pour plate method has a limit of quantification (LOQ) of 10^5 c.f.u./kg sample which is well below the target application range in premixtures and feedingstuffs. This method is recommended for official control.

The procedure for enumerating colony forming yeast cells in feedingstuffs involves a reconstitution of test samples in diluent followed by homogenisation of this suspension. To prepare the initial suspension 50 g sample are homogenised in phosphate buffered saline. Decimal serial dilutions are prepared in peptone salt diluent from the initial suspension. An aliquot of 1 ml of appropriate decimal dilutions is transferred into chloramphenicol yeast glucose extract agar pour plates which are incubated at 35 ºC for 2 days for determination of the final colony count.

Concerning the unambiguous identification of the specific strain of *S. cerevisiae* NCYC Sc47 in premixtures and feedingstuffs, a polymerase chain reaction (PCR) method is recommended for official control purposes [5]. This method was validated in a collaborative study which demonstrated a high level of correct identification between laboratories [6].

4. CONCLUSIONS AND RECOMMENDATIONS

Concerning the enumeration of the active agent of BIOSAF® Sc47 (*Saccharomyces cerevisiae* NCYC Sc47) in the *additive* BIOSAF® Sc47, the ISO 7954 Standard pour plate method is appropriate [3]. For official control of the active agent in the *additive*, *premixtures* and *feedingstuffs* the CRL recommends – however - a pour plate method [4] which is based on the ISO Standard and which has been validated on the target matrix (feedingstuffs) by an interlaboratory study.

Concerning the unambiguous identification of the specific strain of *S. cerevisiae* NCYC Sc47 in BIOSAF® Sc47, and if necessary in *premixtures* and *feedingstuffs*, a polymerase chain reaction (PCR) method is used [5]. This method was validated in a collaborative study [6] on the target matrix and demonstrated a high level of correct identification between laboratories, and is therefore recommended for official control.

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

5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

In accordance with the requirements of Regulation (EC) No 1831/2003, samples of BIOSAF® Sc47 for small ruminants 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] Annex III Proposal of Register entry

[2] Dossier Section V Monograph, 2.2 Biological origin

[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. RAPPORTEUR LABORATORY

The Rapporteur Laboratory for this evaluation was the Community Reference Laboratory for Feed Additive Authorisation (CRL-FAA), Geel, Belgium.