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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-2006-067

Name of Additive: BIOSAF® Sc47 (calves for rearing)

Active Agent(s): *Saccharomyces cerevisiae* NCYC Sc47

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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. The active agent in the additive is a strain of *Saccharomyces cerevisiae* NCYC Sc47. The additive represents heat resistant micro-granules containing live yeast cells with a minimum concentration of 5×10^9 colony forming units (c.f.u.) per gram. Specifically, authorisation is sought to use BIOSAF[®] Sc47 for calves for rearing. The conditions of use are proposed with a recommended dosage of 1.5×10^9 to 2.0×10^{10} c.f.u./kg complete feedingstuffs.

For the quantification of the active agent (*Saccharomyces cerevisiae* NCYC Sc47) of BIOSAF[®] Sc47 in the *feed additive* an appropriate pour plate method (ISO 7954) using a yeast extract agar was proposed by the applicant. For an analysis of the active agent in *premixtures* and *feedingstuffs* a ring-trial validated pour plate method using chloramphenicol glucose yeast extract (CGYE) agar (Leuschner R.G.K. et al., 2003. System. Appl. Microbiol. 26, 147-153) is suggested. The method performance characteristics of the enumeration method include relative standard deviations for within-laboratory repeatability (RSD_r) and between-laboratory reproducibility (RSD_R) of 2 to 5 % and of around 8 %, respectively. For official controls of *premixtures* and *feedingstuffs* supplemented with BIOSAF[®] Sc47 this ring-trial validated method using CGYE agar is recommended. The limit of quantification (LOQ) for the method is around 2 to 3×10^5 c.f.u./kg sample which is well below the minimum target level of application in feedingstuffs.

The identity of the bacterial strain, *Saccharomyces cerevisiae* NCYC Sc47, was analysed by polymerase chain reaction (PCR) and pulsed field gel electrophoresis (PFGE). The PCR method was ring-trial validated and performed appropriately (Leuschner R.G.K. et al., 2004. System. Appl. Microbiol. 27, 492-500). The PCR method is considered suitable for official controls in the frame of the authorisation.

Supplementary experimental work is not considered necessary based on the documentation provided.

KEYWORDS

BIOSAF[®] Sc47, feed additive, *Saccharomyces cerevisiae*, zootechnical additive, calves

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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 micro-granules supplemented with a strain of *Saccharomyces cerevisiae*. The strain is deposited as *Saccharomyces cerevisiae* NCYC Sc47 at the National Collection of Yeast Cultures (NCYC), Norwich, UK [1]. BIOSAF[®] Sc47 contains *Saccharomyces cerevisiae* NCYC Sc47 at least at a concentration of 5×10^9 c.f.u. per gram feed additive [2]. The intended use of the current application (EFSA-Q-2006-067) is for calves for rearing. The proposed conditions of use for calves for rearing are a recommended dosage of $1.5 \times 10^9 - 2.0 \times 10^{10}$ c.f.u./kg complete feedingstuffs [2, 3].

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-2006-067) 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 concentration of the active agent, *Saccharomyces cerevisiae* NCYC Sc47, in colony forming units (c.f.u.) per gram additive is at least 5×10^9 c.f.u./g [1]. The method for quantifying the active agent as proposed by the applicant is a pour plate method using a yeast extract agar according to ISO 7954 [4]. The results are reported as colony forming units (c.f.u.) per gram (g) additive. A sample is suspended in a dilution buffer and decimally diluted. Appropriate dilutions are added to yeast extract agar, mixed and transferred into Petri dishes for settling. The agar plates are incubated at 25 °C for 3, 4 or 5 days. A similar pour plate method which uses chloramphenicol glucose yeast extract (CGYE) agar was validated by a collaborative study [5]. The inoculated CGYE agar plates were incubated at 35 °C for 2 days. The fully ring-trial validated method is recommended for official controls in the frame of the authorisation.

The unique characteristics of the strain are examined by a combination of techniques. The production strain was investigated biochemically by determining its fermentation spectrum. The genetic identity of the strain was analysed using molecular methods such as polymerase chain reaction (PCR) and pulsed field gel electrophoresis (PFGE). The applicant proposes a PCR method for analysis which was developed to unambiguously identify yeast strains [6]. This method was validated in a collaborative study and performed appropriately amongst laboratories [7]. The PCR method is recommended for official controls in the frame of the authorisation.

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)

For the enumeration of the active agents *Saccharomyces cerevisiae* NCYC Sc47 in *premixtures* and *feedingstuffs*, the applicant proposes the above mentioned ring-trial validated pour plate method [5]. The method was validated in a collaborative study and satisfactory performance characteristics were obtained, since the RSD_T was between 2 to 5 % and the RSD_R around 8 %. This method is recommended for official controls in the frame of the authorisation.

For the identification of the strain of *Saccharomyces cerevisiae* NCYC Sc47 in animal feed the same PCR method as for the analysis of the feed additive is proposed [7]. The PCR analysis was carried out on individual yeast colonies isolated from agar plates. The validated PCR method is recommended for official controls in the frame of this authorisation.

4. CONCLUSIONS AND RECOMMENDATIONS

The applicant uses appropriate conventional methods to enumerate the active agent in the *feed additive* and in *feedingstuffs*. A ring-trial validated method using CGYE agar is recommended for official controls in the frame of the authorisation [5].

For analysis of the identity of the bacterial strain, *Saccharomyces cerevisiae* NCYC Sc47, uses the applicant a range of techniques which are appropriate to identify the strain. A suggested and validated PCR method is recommended for official controls in the frame of this authorisation [7].

Supplementary experimental work is not considered necessary based on the documentation provided.

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

Analytical method suitable for official controls: Pour plate method using agar chloramphenicol glucose yeast extract agar based on ISO 7954. 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 calves for rearing have been sent to the Community Reference Laboratory for Feed Additives Authorisation. The dossier has been made available to the CRL by EFSA.

7. REFERENCES

- [1] Technical dossier, section II, 1. Identity of the additive
- [2] Public Summary of the dossier
- [3] EFSA, Annex III Proposal of Register entry
- [4] ISO 7954. 1987. General guidance for enumeration of yeasts and moulds – Colony count technique at 25 °C.
- [5] Leuschner R.G.K., Bew J., Bertin G. 2003. Validation of an official control method for enumeration of authorised probiotic yeast in animal feed. System. Appl. Microbiol. 26, 147-153.
- [6] Nes F., Lavallée, Dubourdieu D., Aigle M., Dulau L. J. 1993. Identification of yeast strains using the polymerase chain reaction. Sci. Food Agric. 62, 89-94.
- [7] Leuschner R.G.K., Bew J., Fourcassier P., Bertin G. 2004. Validation of the official control method based on polymerase chain reaction (PCR) for identification of authorised probiotic yeast in animal feed. System. Appl. Microbiol. 27, 492-500.

8. RAPPORTEUR LABORATORY

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