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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-2005-149

Name of Additive: BIOSAF® Sc47 (for lambs)

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

Rapporteur Laboratory: Community Reference Laboratory for  
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## EXECUTIVE SUMMARY

In the current application authorisation is sought for BIOSAF<sup>®</sup> 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<sup>®</sup> Sc47 as a growth performance enhancer for lambs. BIOSAF<sup>®</sup> Sc47 is a micro-granular product. The feed additive is proposed for use in feedingstuffs or feed supplements during the entire life of lambs at a final concentration of  $10^6 - 10^7$  colony forming units (c.f.u.) per gram (g) feedingstuff.

For the enumeration of the active agent (*Saccharomyces cerevisiae* NCYC Sc47) in the *feed additive*, a pour plate standard method using a chloramphenicol yeast extract agar, namely the ISO 7954 is proposed by the applicant. This method is considered appropriate for the field of application that is sought and is considered appropriate for official control purposes.

For the enumeration of the active agent, *S. cerevisiae* NCYC Sc47 in *premixtures* and *feedingstuffs*, a pour plate method based on the above ISO method is proposed by the applicant. The proposed method was validated in an inter-laboratory comparison [Appl. Microbiol., 26, 147-153] for which performance characteristics include relative standard deviations for repeatability ( $RSD_r$ ) and for between-laboratory reproducibility ( $RSD_R$ ) of around 5 % and 8 %, respectively. The limit of quantification (LOQ) for the method is 100 c.f.u./g. These performance characteristics are considered acceptable and the method is therefore considered suitable for official control for the active agent in premixtures and feedingstuffs.

For identification of the *Saccharomyces* strain a polymerase chain reaction (PCR) method is proposed [ J. Sci. Food Agric., 62, 89-94]. The method was validated in an inter-laboratory study [System. Appl. Microbiol. 27,492-500], and is considered appropriate for official control purposes in the feed additive, premixtures and feedingstuffs.

Further testing or validation of the submitted methods for the enumeration and identification of the active ingredient are not considered necessary.

## **KEYWORDS**

BIOSAF<sup>®</sup> Sc47, yeast, zootechnical, *Saccharomyces cerevisiae*, feed additive

## **TABLE OF CONTENTS**

1. BACKGROUND.....	3
2. TERMS OF REFERENCE.....	3
3. EVALUATION.....	4
4. CONCLUSIONS AND RECOMMENDATIONS.....	6
5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL.....	7
6. REFERENCES.....	7
7. RAPPORTEUR LABORATORY.....	7

### **1. BACKGROUND**

BIOSAF<sup>®</sup> Sc47 is a feed additive for which authorisation is sought under the ‘zootechnical additives’, functional group ‘other zootechnical additives’, according to Annex I of Regulation (EC) No 1831/2003.

It contains yeast cells of the strain of *Saccharomyces cerevisiae* NCYC Sc47 as the active agent. BIOSAF<sup>®</sup> Sc47 is already authorised for fattening bovine feedingstuff, approved, in 2003, for permanent authorisation (Regulation (EC) No 316/2003).

The intended use (*cf.* EFSA-Q-2005-149) of the current application is to enhance the growth performance of lambs, by mixing the feed additive into compound feedingstuffs at a concentration of 1 – 10 grams per kg feedingstuff.

### **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-FAA is requested to submit a full evaluation report to the European Food Safety Authority (EFSA) for each application. For this

particular dossier, the suitability of the methods of analysis and validation studies submitted in connection with, *cf.* EFSA-Q-2005-149, was evaluated.

### 3. EVALUATION

The numbering system under this point refers to that of the Guidelines for the assessment of additives in feedingstuffs, PART II: Enzymes and Micro-organisms (the Guidelines). The method protocols and corresponding validation data are given in Section II of the dossier whereas the method protocol for the determination of residues in animal tissue is specified in another file of the dossier.

*Description of the methods used for the determination of the criteria listed under items 2.1.3, 2.1.4, 2.1.5, 2.2.5, 2.2.6, 2.3.1, 2.3.2 and 2.3.3 of the Guidelines.*

*Qualitative and quantitative composition of the additive BIOSAF<sup>®</sup> Sc47*

The *active agent* is a strain of the yeast *Saccharomyces cerevisiae* (NCYC Sc47) which is deposited at the National Collection of Yeast Culture (UK). The applicant proposes a standard pour plate method using chloramphenicol yeast extract agar for enumeration, namely the ISO 7954 method[1]. The number of viable microorganisms is given in colony forming units (c.f.u.) per g. The method is considered suitable for official control purposes in the sought field of application.

*Identification and characterisation*

For unambiguous *identification* of the strain *Saccharomyces cerevisiae* NCYC Sc47 in the additive, premixtures or feedingstuffs, a published polymerase chain reaction (PCR) method was used [3] which was validated in an inter-laboratory study [4] which demonstrated a high level of correct identification between laboratories. This method is considered appropriate for official control purposes.

(*Cf.* the requirements listed in point 2.1.3 of the Guidelines.)

### *Stability of the additive*

The applicant used the same pour plate method as mentioned above for enumeration of the active agent in stability studies of BIOSAF<sup>®</sup> Sc47 [1]. To demonstrate genetic stability of the *S. cerevisiae* NCYC Sc47 strain, the same PCR method as mentioned above is proposed by the applicant [3, 4]. The methods used for the stability studies are considered appropriate (*Cf.* the requirements listed in point 2.3.1 of the Guidelines).

### ***Description of the qualitative and quantitative methods for routine control of the active agent in premixtures and feedingstuffs***

To determine the presence of the active agent in premixtures and feedingstuffs, the applicant proposes a pour plate method based on ISO 7954 [1], using chloramphenicol glucose yeast extract agar. The method was validated in an inter-laboratory study [2]. An internationally accepted protocol for such studies was applied [5]. Performance characteristics included relative standard deviations for repeatability ( $RSD_r$ ) and relative standard deviations for between-laboratory reproducibility ( $RSD_R$ ) of around 5 % and 8 %, respectively, as well as a LOQ of 100 c.f.u./g. All of these values are considered acceptable.

In addition, a plate count method using CHROMagar Candida, an agar where colonies of the species *S. cerevisiae* appear in a purple colour, is proposed by the applicant. The chromogenic agar was validated in an inter-laboratory study in parallel with the above mentioned pour plate method [2], and resulted in  $RSD_r$  and between-laboratory  $RSD_R$  values of about 3 % and 6 % respectively, as well as a LOQ of 1000 c.f.u./g, all of which are considered acceptable. However, the use of the chromogenic agar, as specified in the plate count method, is not as commonly used as the pour plate method.

Therefore, depending on the specific purpose of the analysis, the pour plate method would be the method of choice for official control purposes.

(*Cf.* the requirements listed in point 2.5.2 of the Guidelines).

### CHECKLIST FOR METHODS SUBMITTED

		Yes	No	N/A
1.1	Is/Are the method(s) mentioned on Premixtures accompanied by information on:			<b>X</b>
1.2	Is/Are the method(s) mentioned on Feedingstuffs accompanied by information on:			
	- Sampling method used	<b>X</b>		
	- Specificity	<b>X</b>		
	- Accuracy	<b>X</b>		
	- Precision	<b>X</b>		
	- Limits of quantification	<b>X</b>		
	- Validation procedure used	<b>X</b>		

N/A: Not applicable

## 4. CONCLUSIONS AND RECOMMENDATIONS

Concerning the enumeration of the active agent *Saccharomyces cerevisiae* NCYC Sc47 in the additive, the applicant proposes a pour plate method, the standard method ISO 7954 [1]. The ISO method is considered appropriate for the purpose of official control.

Concerning the enumeration of the active agent in premixtures and feedingstuffs, a pour plate count method that has been published in a peer reviewed journal is suggested. This method is based on the principles of the above-mentioned ISO 7954 method and was fully validated [2] applying an internationally accepted protocol for inter-laboratory studies [5]. Performance characteristics for this method were expressed in terms of RSD<sub>T</sub> and RSD<sub>R</sub> values of around 5 % and 8 %, respectively, and a LOQ of 100 c.f.u. per gram, all of which are considered acceptable. Alternatively, in cases where the species *S. cerevisiae* needs to be confirmed, a validated plate count method using an elective chromogenic agar was proposed [2]. Both methods are considered appropriate for official control purposes.

Concerning the unambiguous identification of the specific strain of *S. cerevisiae* NCYC Sc47 in the additive, premixtures and feedingstuffs, a polymerase chain reaction (PCR) method was used [3]. This method was validated in a inter-laboratory study [4] which demonstrated a high level of correct identification between laboratories, and it is therefore considered suitable for official control purpose as well.

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

## **5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL**

In accordance with the requirements of Regulation (EC) No 1831/2003, samples of BIOSAF® Sc47 have been sent to the CRL-FAA. The dossier has been made available to the CRL-FAA by EFSA.

## **6. REFERENCES**

- [1] Anonymous: ISO 7954, 1987. General guidance for enumeration of yeasts and moulds – Colony count technique at 25 °C
- [2] 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
- [3] 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
- [4] 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
- [5] Horwitz W. 1995. Protocol for the design, conduct and interpretation of method-performance studies. *Pure Appl. Chem.* 76, 331-343

## **7. RAPPORTEUR LABORATORY**

The Rapporteur Laboratory for this evaluation was the CRL-FAA, EC-JRC-IRMM, Geel, Belgium.