



D08/FSQ/CVH/RL/D(2008) 23555

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-2008-009
FAD-2007-0048

Name of Additive: YEA-SACC^{1026®}

Active Agent(s): *Saccharomyces cerevisiae* CBS 493.94

Rapporteur Laboratory: Community Reference Laboratory for
Feed Additives (CRL-FA)

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Date: 16/09/2008

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Date: 16/09/2008

EXECUTIVE SUMMARY

In the current application authorisation is sought for the microbial feed additive Yea-Sacc^{1026®} under the category 'zootechnical additives', functional groups 'digestibility enhancers and gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003. Specifically, the use of YEA-SACC^{1026®} for horses is requested. YEA-SACC^{1026®} contains a minimum of 1×10^9 viable cells (c.f.u., colony-forming units) of *Saccharomyces cerevisiae* CBS 493.94 (as active agent) per gram. The feed additive is intended to be mixed into complete feedingstuffs at a final concentration of 4.0×10^8 to 2.5×10^{10} c.f.u./kg.

For the determination of the active agent, *Saccharomyces cerevisiae* CBS 493.94, 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* CBS 493.94 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 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 1000 colony forming units (c.f.u) per gram (g) feed additive or premixture and 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

YEA-SACC^{1026®}, yeast, zootechnical, *Saccharomyces cerevisiae*, horses

1. BACKGROUND

YEA-SACC^{1026®} is a feed additive for which authorisation is sought under the category 'zootechnical additives', functional groups 'digestibility enhancers and gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003. YEA-SACC^{1026®} is provided in form of a powder/prills containing at least 5×10^9 c.f.u. viable cells of *Saccharomyces cerevisiae* CBS 493.94 per gram as active agent. The strain is deposited at the National Collection of Yeast Cultures (NCYC) in Norwich, UK and registered as *Saccharomyces cerevisiae* NCYC¹⁰²⁶ [1]. The intended use of the current application are feedingstuffs for horses, pre- and post-weaning foal (whole life of the animal), by mixing the feed additive into complete feedingstuffs at a final concentration of 4.0×10^8 to 2.5×10^{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 YEA-SACC^{1026®} dossier (EFSA-Q-2008-009) 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 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 was fully ring-trial validated and is recommended for official controls in the frame of the authorisation [4].

The active agent is a strain of the yeast *Saccharomyces cerevisiae* (CBS 493.94). For identification of the authorised strain *Saccharomyces cerevisiae* CBS 493.94 a published polymerase chain reaction (PCR) method was used [5]. The method was validated by a collaborative study [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 applicant proposes the same method as above [4] to analyse premixtures and feedingstuffs for YEA-SACC^{1026®} [7]. 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 1000 colony forming units (c.f.u) per gram (g) additive or premixtures and 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* CBS 493.94 in YEA-SACC^{1026®} and in premixtures and feedingstuff supplemented with YEA-SACC^{1026®}, 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 *Saccharomyces cerevisiae* CBS 493.94 in the feed additive, premixtures and feedingstuffs.

The proposed methods are ring-trial validated and are considered appropriate for official controls of the active agent expressed in c.f.u..

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 YEA-SACC^{1026®} for horses 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, 2.2.1.4. Origin of strain
- [2] Proposal of Register entry Annex III
- [3] ISO 7954. 1987. General guidance for enumeration of yeasts and moulds – Colony count technique at 25 °C
- [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 II, 2.5.2. Description of the qualitative and quantitative methods for routine control of the active agent in premixtures and feedingstuffs

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

The following National Reference Laboratories contributed to the initial report:

- Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, Oberschleißheim, Germany
- Thüringer Landesanstalt für Landwirtschaft, Jena, Germany
- National Feed Laboratory, Lublin, Poland