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Community Reference Laboratory for Feed Additives

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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-2007-139 FAD-2007-0029
Name of Additive:	LEVUCELL® for lambs
Active Agent(s):	*Saccharomyces cerevisiae* CNCM I-1077
Rapporteur Laboratory:	Community Reference Laboratory for Feed Additives (CRL-FA)
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Report approved by:	Christoph von Holst (CRL-FA)
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EXECUTIVE SUMMARY

In the current application authorisation is sought for LEVUCELL® SC under the category 'zootechnical additives', functional group 'gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003. Specifically, the use of LEVUCELL® SC for lambs in its two forms LEVUCELL® SC20 and LEVUCELL® SC 10ME is requested. LEVUCELL® SC20 (non-coated form) contains at least 20 x 10^9 of viable cells (c.f.u., colony forming units) of *Saccharomyces cerevisiae* CNCM I-1077 as active agent per gram and LEVUCELL® SC10ME (coated form) contains at least 10 x 10^9 c.f.u./g, respectively. The feed additive is intended to be mixed into complete feedingstuffs for lambs at a final concentration of 3 to 7.3 x 10^9 c.f.u./kg.

For the determination of the active agent (*Saccharomyces cerevisiae* CNCM I-1077) in the *feed additive* LEVUCELL® SC, a pour plate method for both forms of the additive and a polymerase chain reaction (PCR) method for identification are proposed which are considered appropriate for the intended purpose. The method was shown to be applicable for the coated and uncoated form of the active agent in the feed additive.

For official controls of the active agent *S. cerevisiae* CNCM I-1077 in the *feed additive*, *premixtures* and *feedingstuffs*, a similar ring-trial validated pour plate method that is applicable for the feed additive is proposed. The method's performance characteristics 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 [System. Appl. Microbiol. 2003, 26, 147-153]. The method was shown to be equally applicable for the coated and uncoated form of the active agent in the feed additive, premixtures and is therefore expected to perform similarly in feedingstuffs with regards to the coating. The pour plate method has a limit of quantification (LOQ) of 10^5 c.f.u./kg sample.

A PCR method for strain identification which performed appropriately in a ring-trial validation study [System. Appl. Microbiol. 2004, 27, 492-500] is recommended for official controls for the field of application sought.

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

KEYWORDS

LEVUCELL® SC, feed additive, yeast, lambs, zootechnical, *Saccharomyces cerevisiae*

1. BACKGROUND

LEVUCELL® SC 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. LEVUCELL® SC is provided in two forms, an uncoated form LEVUCELL® SC20 and a coated (encapsulated) form LEVUCELL® SC10ME both of which contain viable cells of the yeast strain *Saccharomyces cerevisiae* CNCM I-1077 as the active agent. LEVUCELL® SC20 contains at least 20 x 10^9 [1] of viable cells (c.f.u., colony forming units) of *Saccharomyces cerevisiae* CNCM I-1077 as active agent per gram and LEVUCELL® SC10ME contains at least 10 x 10^9 c.f.u./g [1,2]. The yeast strain is deposited at the Collection Nationale de Cultures de Microorganismes (C.N.C.M.) at the Institut Pasteur, Paris, France. The intended use of the current application is for lambs, by mixing the feed additive into feedingstuffs at a proposed final concentration of 3 to 7.3 x 10^9 c.f.u./kg complete 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 (EFSA) for each application. For this particular dossier, the methods of analysis submitted in connection with the LEVUCELL® SC dossier (EFSA-Q-2007-139) 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 quantifying the active agent in the additive as provided by the applicant represents a validated pour plate method using chloramphenicol glucose yeast extract (CGYE) agar which is considered acceptable for official controls [3]. The applicant provided scientific evidence that the method is applicable to both forms of the additive including the encapsulated form [4]

The active agent is a strain of the yeast *Saccharomyces cerevisiae* (CNCM I-1077). The physiological and biochemical properties of the strain are appropriately characterised by using an accepted commercially available API 20C gallery. For identification of the authorised strain of *Saccharomyces cerevisiae* CNCM I-1077, a range of biochemical and DNA based techniques are used including a ring-trial validated and published polymerase chain reaction (PCR) method [5,6]. This method is considered appropriate for official controls.

Qualitative and quantitative composition of any impurities in the additive

The applicant uses a range of methods for potential impurities in the additive [7]. The applicant provided quality control results for contents of heavy metals including arsenic (As), cadmium (Cd), chromium (Cr), mercury (Hg), nickel (Ni), tin (Sn), lead (Pb) and selenium (Se) using an atomic adsorption method as described in the European Pharmacopeia, 2nd Edition, V. 6.17. The method is considered suitable for official controls.

For aflatoxins B_1, B_2, G_1 and G_2 the applicant proposed an EU method (Directive 76/372/EEC) which contains a validation for analysis of aflatoxin B_1. It may be considered equally appropriate for aflatoxins B_2, G_1 and G_2. Zearalenone was analysed by a standard method (AFNOR standard, NF V 18-201; ISO 6870). For the analysis of ochratoxin A the method described by the Association of the Analytical Community International (AOAC, 1984, 26, 494) was used. The methods described are considered fit for the purpose of official control.

Microbiological quality of the additive was ensured by investigating coliforms, pathogenic staphylococci, sulphite reducing bacteria, *Clostridium perfringens* and *Salmonella* species. For the determination of mesophilic bacteria tryptone agar was used (NF ISO 4833). Coliforms were determined at 44 ºC using crystal violet red bile lactose medium (NF ISO 4832). Staphylococci were enumerated on an appropriate selective agar (NF ISO 6888).

Sulphite reducing anaerobic bacteria were determined using tryptone sulphite cycloserine (TSC) medium. *Clostridium perfringens* was determined using tryptone sulphite cycloserine (TSC) medium (NF ISO 7937). The detection of *Salmonella* species was carried out in accordance with the standard method NF ISO 6579. The standard methods used for analyses are considered appropriate for official controls for the field of application sought.

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 premixtures and feedingstuffs for the active agent of LEVUCELL® SC, the applicant proposes a method that was published and validated by a full collaborative study using feedingstuffs [3]. The pour plate method using chloramphenicol glucose yeast extract (CGYE) agar would be recommended for analysis. The method's performance characteristics 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 and would be considered acceptable for official controls. The applicant provided data to show the applicability of the method to the coated and uncoated form of the active agent in premixtures and based on the results applicability with regards to the coated form in feedingstuffs is concluded [4]. The pour plate method has a limit of quantification (LOQ) of 10^5 c.f.u./kg sample. The method is recommended for official controls.

Concerning the unambiguous identification of the specific strain of *S. cerevisiae* CNCM I-1077 in the additive LEVUCELL® SC a polymerase chain reaction (PCR) method is used [5], which can also be applied to premixtures and feedingstuffs. This method was validated in a collaborative study using feed samples which demonstrated a high level of correct identification between laboratories [6]. The PCR method is considered suitable for official controls for the field of application that is sought.

4. CONCLUSIONS AND RECOMMENDATIONS

Concerning the enumeration of the active agents of LEVUCELL® SC (*Saccharomyces cerevisiae* CNCM I-1077) in the *additive*, in *premixtures* and *feedingstuffs*, the applicant

proposes a ring-trial validated pour plate method using chloramphenicol glucose yeast extract (CGYE) agar [3]. The method is considered appropriate for official controls.

Concerning the unambiguous identification of the specific strain of *S. cerevisiae* CNCM I-1077 in the additive LEVUCELL® SC a polymerase chain reaction (PCR) method is used [4], which can also be applied to premixtures and feedingstuffs. 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].

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 LEVUCELL® SC for lambs 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] Proposal of Register entry Annex III
[2] Technical dossier, Section II, chapter 1.3.2.2 " Quantitative composition of the active substance expressed in CFU/g"
[3] 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
[4] Confirmatory letter and scientific data presentation of applicant. 2005. Entitled 'Examples of repeatability and reproducibility tests performed using the method described by Leuschner et al., 2003' (initially submitted with the dossier LEVUCELL for horses, FAD-2005-0018)
[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, 5. Control methods

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was the Community Reference Laboratory for Feed Additives (CRL-FA), Geel, Belgium. This initial evaluation report was made available for commenting to the consortium of National Reference Laboratories.

8. ACKNOWLEDGEMENTS

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- Laboratory Agroalimentari, Catalonia, Spain