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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-2005-176

Name of Additive: LEVUCCELL[®]SC for dairy goats and dairy
ewes

Active Agent(s): *Saccharomyces cerevisiae* CNCM I-1077

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EXECUTIVE SUMMARY

In the current application authorisation is sought for LEVUCCELL[®] SC under the category ‘zootechnical additives’, according to Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought to use LEVUCCELL[®] SC in its two forms LEVUCCELL[®] SC 20 and LEVUCCELL[®] SC 10ME for dairy goats and dairy ewes. LEVUCCELL[®] SC is a light brown powdery product whereby LEVUCCELL[®] SC 20 is uncoated and LEVUCCELL[®] SC 10ME micro-encapsulated. The feed additive is proposed for use in premixtures and feedingstuffs for dairy goats at a minimum concentration of 4.8×10^8 and maximum concentration of 2.9×10^9 colony forming units (c.f.u.) per kg complete feedingstuffs and for dairy ewes at a concentration of 1.2×10^9 c.f.u./kg complete feedingstuffs.

For the determination of the active agent (*Saccharomyces cerevisiae* CNCM I-1077) in the *feed additive* LEVUCCELL[®] SC, a pour plate method and a molecular identification method (polymerase chain reaction (PCR)) are proposed, which are considered appropriate for the intended purpose.

For the determination of the active agent *S. cerevisiae* CNCM I-1077 in *premixtures and feedingstuffs*, the same methods as for the additives are proposed. The method’s performance characteristics of the enumeration method include relative standard deviations for repeatability (RSD_r) and between-laboratory reproducibility (RSD_R) of around 5 % and 8 %, respectively [System. Appl. Microbiol. 2003, 26, 147-153]. The limit of quantification (LOQ) of the method is 100 c.f.u./g. These performance characteristics are considered acceptable. The PCR method for strain identification was ring trial validated and performed appropriately. Both methods are considered suitable for official control for the field of application that is sought [System. Appl. Microbiol. 2004, 27, 492-500].

Official and/or standard methods are proposed by the applicant for the determination of impurities (heavy metals, mycotoxins, microbiological quality) in the feed additive. The methods are therefore suitable for official control purposes.

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

KEYWORDS

LEVUCCELL[®] SC, feed additive, yeast, goats, ewes, zootechnical, *Saccharomyces cerevisiae*

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1. BACKGROUND

LEVUCCELL[®] SC is a feed additive for which authorisation is sought under the category ‘zootechnical additives’, according to Annex I of Regulation (EC) No 1831/2003. It contains viable cells of the yeast strain *Saccharomyces cerevisiae* CNCM I-1077 as active agent. The yeast strain is deposited at the Collection Nationale de Cultures de Microorganismes (C.N.C.M.) at the Institut Pasteur, Paris, France. LEVUCCELL[®] SC is presented in two different forms:

- LEVUCCELL[®] SC 20 containing 20×10^9 c.f.u. revivable dried cells of *S. cerevisiae* CNCM I-1077 per gram of additive
- LEVUCCELL[®] SC 10ME containing 10×10^9 c.f.u. revivable dried cells of *S. cerevisiae* CNCM I-1077 per gram microencapsulated product (50 % LEVUCCELL[®] SC 20 and 50 % fatty acid blend)

LEVUCCELL[®] SC is already authorised as feed additive for dairy cows and cattle for fattening (Regulation EC No.1200/2005). The intended use (*cf.* EFSA-Q-2005-176) of the current application is to enhance milk yield and composition of dairy goats and dairy ewes, by mixing the feed additive into compound feedingstuffs at a proposed final concentration of 4.8×10^8 to 2.9×10^9 c.f.u./kg for dairy goats and at a final concentration of 1.2×10^9 c.f.u./kg for dairy ewes.

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-2005-176, 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 method protocols and corresponding validation data – if applicable – 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 some of the methods listed under items 2.5.1 of the Guidelines.

Qualitative and quantitative composition of the additive LEVUCCELL[®] SC

The additive LEVUCCELL[®] SC 20 is a fine, granulated, light brown powder which flows easily and has an odour similar to baker's yeast. It is composed of approximately 80 % revivable *Saccharomyces cerevisiae* CNCM I-1077 dried yeast cells and approximately 14 % non-viable yeast cells which lost their viability during the drying process. About 1 % represents sorbitane monostearate (E 491) and a rest moisture content of 5 % remains in the additive. LEVUCCELL[®] SC 10ME is composed of 50 % LEVUCCELL[®] SC 20 and 50 % stearic/palmitic acids blend (Exaflor). The concentrations of the active agents in colony forming units (c.f.u.) per gram in both forms of the additive LEVUCCELL[®] SC are described above (background section).

The method for quantifying the active agent in the additive as provided by the applicant represents a pour plate method using chloramphenicol glucose yeast extract (CGYE) agar which is considered suitable for the intended purpose [1].

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 DNA based techniques are used such as polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP) and pulsed field gel electrophoresis (PFGE). For a RFLP method based on mitochondrial polymorphism a rapid minimal-preparation method is used according to the applicant, however the method protocol or performance data are not provided. A ring-trial validated and published polymerase chain reaction (PCR) method was also used [2, 3]. This method is considered appropriate for official control purposes.

Qualitative and quantitative composition of any impurities in the additive

The applicant provided quality control results and copies of a short description of the method of analysis for heavy metals including arsenic (As), cadmium (Cd), chromium (Cr), mercury (Hg), nickel (Ni), tin (Sn) and selenium (Se) using an atomic adsorption method as described in the European Pharmacopoeia, 2nd Edition, V. 6.17, 2.2.22. The method is considered suitable for official control purposes.

For aflatoxins B₁, B₂, G₁ and G₂ the applicant proposed an EU method (Directive 76/372/EEC) which contains a validation for analysis of aflatoxin B₁. It may be considered equally appropriate for aflatoxins B₂, G₁ and G₂. 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 was ensured by investigating aerobic mesophilic bacteria, 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 °C 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. Copies of the protocols for the standard methods were provided by the applicant without any corresponding method performance data for the field of application. The standard methods are however considered appropriate for the purpose of official control for the field of application that is sought.

Description of the qualitative and quantitative methods for routine control of the active agent in premixtures and feedingstuffs (cf. requirements in guidelines section 2.5.2)

To analyse premixtures and feedingstuffs for the active agent of LEVUCCELL[®] SC, the applicant proposes a method that was published and validated by a full collaborative study [1]. The pour plate method using chloramphenicol glucose yeast extract (CGYE) agar would be recommended for analysis. Performance characteristics of this method obtained in the collaborative study were expressed in terms of relative standard deviations for repeatability (RSD_r) and reproducibility (RSD_R) which were around 5 % and 8 %, respectively and would be considered acceptable for official control purposes. The pour plate method has a limit of quantification (LOQ) of 100 c.f.u./g and the plate count method a quantification limit (LOQ) of 1000 c.f.u./g both of which are well below the target application range.

Concerning the unambiguous identification of the specific strain of *S. cerevisiae* CNCM I-1077 in the additive LEVUCCELL[®] SC a polymerase chain reaction (PCR) method is used [2], 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 [3]. The PCR method is considered suitable for official control purposes for the field of application that is sought.

4. CONCLUSIONS AND RECOMMENDATIONS

Concerning the enumeration of the active agents of LEVUCCELL[®] SC (*Saccharomyces cerevisiae* CNCM I-1077) in the *additive*, in *premixtures* and *feedingstuff*, the applicant

proposes a ring-trial validated pour plate method using chloramphenicol glucose yeast extract (CGYE) agar [1]. Performance characteristics of this method obtained in the collaborative study were expressed in terms of relative standard deviations for repeatability (RSD_r) and reproducibility (RSD_R) which were around 5 % and 8 %, respectively. Both values are considered acceptable. The limit of quantification (LOQ) of this method is 100 colony forming units (c.f.u) per gram (g) sample, i.e. well below the target application level in feedingstuffs. The method is considered appropriate for official control purposes.

Concerning the unambiguous identification of the specific strain of *S. cerevisiae* CNCM I-1077 in the additive LEVUCCELL[®] SC a polymerase chain reaction (PCR) method is used [2], 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 control purposes [3].

Official and/or standard methods are proposed by the applicant for the determination of impurities (heavy metals, mycotoxins, microbiological quality) in the feed additive. The methods are therefore suitable for official control purposes.

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 LEVUCCELL[®] SC 20 and LEVUCCELL[®] SC ME10 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] 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
- [2] Nes, F., Lavallée F., Dubourdiou D., Aigle M., Dulau L. 1993. Identification of yeast strains using the polymerase chain reaction. J. Sci. Food Agric. 62, 89-94
- [3] 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 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.