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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-2007-165 FAD-2007-0022
Name of Additive:	MycoCell for dairy cows
Active Agent(s):	*Saccharomyces cerevisiae* NCYC R404
Rapporteur Laboratory:	Community Reference Laboratory for Feed Additives (CRL-FA)
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EXECUTIVE SUMMARY

In the current application authorisation is sought for MycoCell under the category 'zootechnical additives', functional groups 'digestibility enhancers', 'gut flora stabilisers', 'other zootechnical additives' according to Annex I of Regulation (EC) No 1831/2003. Specifically, the use of MycoCell for dairy cows is requested. MycoCell is provided in two forms, MycoCell concentrate and MycoCell farm packs which contain at least 5 x 10^9 and 2 x 10^8 to 2 x 10^9 c.f.u. viable cells of the yeast strain *Saccharomyces cerevisiae* NCYC R404 as the active agent per gram, respectively. The feed additive may be effectively used in any feed for dairy cows at a recommended minimum dose of 1 x 10^{10} c.f.u. per day.

For the determination of the active agent (*Saccharomyces cerevisiae* NCYC R404) in the MycoCell concentrate and farm packs, a pour plate method for enumeration is proposed which is considered appropriate for the intended purpose.

For the quantification of the active agent *S. cerevisiae* NCYC R404 in *premixtures* and *feedingstuffs*, the CRL-FA proposes a ring-trial validated method. 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 has a limit of quantification (LOQ) of 10 x 10^5 c.f.u./kg. The CRL considers the method suitable for official control purposes, if the target level is expressed in terms of c.f.u. per *kg feedingstuffs* not in terms of c.f.u. *per day* as specified in the proposed register entry.

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

MycoCell, feed additive, yeast, dairy cows, zootechnical, *Saccharomyces cerevisiae*

1. BACKGROUND

MycoCell is a feed additive for which authorisation is sought under the category ‘zootechnical additives’, functional groups 'digestibility enhancers', 'gut flora stabilisers', 'other zootechnical additives' according to Annex I of Regulation (EC) No 1831/2003. MycoCell is provided in two forms, MycoCell concentrate and MycoCell farm packs which contain at least 5 x 10^9 and 2 x 10^8 to 2 x 10^9 c.f.u. viable cells of the yeast strain *Saccharomyces cerevisiae* NCYC R404 as the active agent per gram, respectively. The yeast strain is deposited at the National Collection of Yeast Cultures (NCYC) at the Institute of Food Research, Norwich, UK. The intended use of the current application is for dairy cows, by mixing the feed additive into feedingstuffs at a proposed minimum dose of 1 x 10^{10} c.f.u. per day [1, 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 MycoCell dossier (EFSA-Q-2007-165) 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 pour plate method using malt extract agar (MEA) which is appropriate for the purpose [4]. For official control a ring-trial validated pour plate method is recommended [6].

The active agent is a strain of the yeast *Saccharomyces cerevisiae* (NCYC R404). The physiological and biochemical properties of the strain are appropriately characterised and for

molecular identification a molecular fingerprint is provided [7]. A ring-trial validated and published polymerase chain reaction (PCR) method is recommended for official controls [8, 9].

Qualitative and quantitative composition of any impurities in the additive

The applicant provided quality control results for contents of heavy metals including arsenic (As), cadmium (Cd), chromium (Cr), mercury (Hg), lead (Pb) and mycotoxins such as aflatoxin, deoxynivalenol, zearalenone, trichothecene T-2, ochratoxin and fumonisin for which the analysis was carried out by an external laboratory and the methods have not been provided [10].

Microbiological quality of the additive was ensured by investigating aerobic bacteria, enterococci, wild yeasts and moulds, coliforms, *Staphylococcus aureus*, presence of *Listeria* species and *Salmonella* species. The applicant used AOAC Int. methods which are considered as appropriate [10]. For official controls internationally recognised standard methods such as International standard Organisation (ISO) and Committee for European Normation (CEN) standard methods where available are recommended in line with current European Community Regulations.

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 MycoCell, the applicant proposes a pour plate method using malt extract agar (MEA) that is considered appropriate [4].

The applicant specifies that MycoCell should be applied daily as a 'top dress' to feedingstuffs or incorporated into total mixed rations [5]. Therefore, the target level of the active agent is expressed in terms of c.f.u. *per day* and not in terms of c.f.u. *per kg feedingstuffs*. Consequently, the requirement for an official control method to *quantify* the active agent in feedingstuffs does not seem to be relevant. Nevertheless, in the case that the active agent needs to be quantified in the feedingstuffs the CRL recommends a pour plate method using chloramphenicol glucose yeast extract (CGYE) agar for analysis [6]. 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. The pour plate method has a limit of quantification (LOQ) of 10 x 10^2 c.f.u./g.

The CRL considers the method suitable for official control purposes, if the target level is expressed in terms of c.f.u. *per kg feedingstuffs* in not in terms of *c.f.u. per day* as specified in the proposed register entry.

Concerning the unambiguous identification of the specific strain of *S. cerevisiae* NCYC R404 in the additive MycoCell, a polymerase chain reaction (PCR) method is recommended which can also be applied to premixtures and feedingstuffs [8]. This method was validated in a collaborative study using feed samples which demonstrated a high level of correct identification between laboratories [9]. 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 MycoCell (*Saccharomyces cerevisiae* NCYC R404) in the *additive*, in *premixtures* and *feedingstuffs*, a ring-trial validated pour plate method using chloramphenicol glucose yeast extract (CGYE) agar is considered appropriate for official controls [6].

Concerning the unambiguous identification of the specific strain of *S. cerevisiae* NCYC R404 in the additive MycoCell a polymerase chain reaction (PCR) method is recommended [8], 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 [9].

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

Identification: Polymerase chain reaction (PCR) method

When the target levels of the active agent are expressed in terms of a concentration:
Enumeration: Pour plate method using chloramphenicol glucose yeast extract agar

5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

In accordance with the requirements of Regulation (EC) No 1831/2003, samples of the additive MycoCell for dairy cows have been sent to the Community Reference Laboratory for Feed Additives. The dossier has been made available to the CRL-FA by EFSA.

6. REFERENCES

[1] Proposal of Register entry, Annex III
[2] Technical dossier, section II, 2.2.7. Other relevant properties
[3] Technical dossier, section II, 2.3.1. Stability of the additive
[4] Technical dossier, section II, 2.5 Control methods
[5] Technical dossier, section II, 2.4.2. Zootechnical additive
[6] 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
[7] Technical dossier, Appendix 8, Confidential yeast full strain R404 analysis report
[8] 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
[9] 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
[10] Technical dossier, Section II, 2.1.4. Qualitative and quantitative composition of any impurities and 2.5.1. General 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. The initial evaluation report was made available for commenting to the consortium of National Reference Laboratories.

8. ACKNOWLEDGEMENTS

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