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EURL Evaluation Report on the Analytical Methods submitted in connection with the Application for Authorisation of a Feed Additive according to Regulation (EC) No 1831/2003

Saccharomyces cerevisiae NCYC R404

(FAD-2012-0038; CRL/120021)

European Union Reference Laboratory
Feed Additives

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Dossier related to:	**FAD-2012-0038 - CRL/120021**
Name of Product:	**MycoCell**
Active Agent (s):	***Saccharomyces cerevisiae* NCYC R404**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **Geel, Belgium**
Report prepared by:	**Gilda D'Arco**
Report checked by: Date:	**Piotr Robouch (EURL-FA)** **02/09/2013**
Report approved by: Date:	**Christoph von Holst** **06/09/2013**

EXECUTIVE SUMMARY

In the current application authorization is sought under article 4(1) for *MycoCell* under the category "zootechnical additives", function group 4(b) "gut flora stabilizer", according to Annex I of Regulation (EC) No 1831/2003. The active component of *MycoCell* is a pure culture of the strain *Saccharomyces cerevisiae* NCYC R404 with a minimum concentration of $1x10^{10}$ Colony Forming Unit (CFU)/g. Specifically, the authorization is sought for the use of *MycoCell* for bovine/dairy cows for milk production. The *feed additive* is intended to be incorporated into *feedingstuffs* through *premixtures* mixed to obtain a minimum dose of 5.7 $x10^{8}$ CFU/kg of *feedingstuffs*.

For the enumeration of *Saccharomyces cerevisiae* NCYC R404 in *feed additive*, *premixtures* and *feedingstuffs* the Applicant proposes the ring trial validated CEN pour plate method for the enumeration of yeast probiotic strains (EN 15789), using yeast extract dextrose chloramphenicol agar (CGYE). The performance characteristics of the EN 15789 method reported after logarithmic transformation (CFU) are:

- a repeatability standard deviation (s_r) ranging from 0.17 to 0.36 $\log_{10}$ CFU/g,
- a reproducibility standard deviation (s_R) ranging from 0.55 to 0.60 $\log_{10}$ CFU/g, and,
- a limit of detection (LOD) of $1x10^{5}$ CFU/kg, well below the minimum dose proposed by the applicant.

Based on these performance characteristics the EURL recommends, for official control, the CEN method EN 15789 for the enumeration of *Saccharomyces cerevisiae* NCYC R404 in *feed additive, premixtures* and *feedingstuffs*.

A polymerase chain reaction (PCR) amplification method, a generally recognized standard methodology for microbial identification, was proposed by the Applicant to establish a DNA fingerprinting, to identify the strain *Saccharomyces cerevisiae* NCYC R404 and to test the purity of this strain within the additive. This generally recognized standard methodology for identification of yeasts is recommended by EURL for official control.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.

KEYWORDS
Saccaharomyces cerevisiae NCYC R404, zootechnical additives, gut flora stabilisers, bovine/dairy cows

1. BACKGROUND

In the current application authorisation is sought under article 4(1) for product *MycoCell* under the category 'zootechnical additives', functional group 4(b) 'gut flora stabilisers', according to Annex I of Regulation (EC) No 1831/2003 [1]. The active component of *MycoCell* is a pure culture of the strain *Saccharomyces cerevisiae* NCYC R404 with a minimum concentration of $1x10^{10}$ Colony Forming Unit (CFU)/g [2, 3]. The strain is deposited at the 'National Collection of Yeast Culture (NCYC)' in Norwich, United Kingdom [4]. Specifically, the authorization is sought for the use of *MycoCell* for bovine/dairy cows for milk production at a minimum dose of 5.7 $x10^8$ (CFU)/kg of *feedingstuffs* [2]. *MycoCell* is not intended for use in pelleted or extruded *feedingstuffs* or *premixtures* containing trace elements.

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009, 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 the tasks of the European Union Reference Laboratory concerning applications for authorisations of feed additives, the EURL is requested to submit a full evaluation report to the European Food Safety Authority (EFSA) for each application or group of applications. For this dossier, the methods of analysis submitted in connection with *MycoCell*, and their suitability to be used for official controls in the frame of the authorisation, were evaluated.

3. EVALUATION

Identification /Characterisation of the feed additive

A polymerase chain reaction (PCR) amplification method, a generally recognized standard methodology for microbial identification, was proposed by the Applicant to establish a DNA fingerprinting, to identify the strain *Saccharomyces cerevisiae* NCYC R404 and to test the purity of this strain within the additive. This standard methodology of identification of yeasts is recommended by EURL for official control.

Quantitative composition of impurities in the additive

The Applicant analysed the *feed additive* for microbial contaminants (such as Enterobacteria, *Escherichia coli, Salmonella* and moulds) by using appropriate EN ISO tests [4]. As for undesirable substances (i.e. arsenic, cadmium, mercury, lead, mycotoxins) several internationally recognised standard methods are available at the respective European Union Reference Laboratories, in accordance with Commission Regulation (EC) No 776/2006.

Description of the analytical methods for the determination of active agent in feed additive, premixture and feedingstuffs.

For the enumeration of *Saccharomyces cerevisiae* NCYC R404 in *feed additive*, *premixtures* and *feedingstuffs* the Applicant proposes the ring trial validated CEN pour plate method for the enumeration of yeast probiotic strains (EN 15789).

The sample is suspended in phosphate buffered saline (PBS) and diluted in a peptone salt solution. The appropriate dilutions are transferred to Petri dishes and a melted CGYE (yeast extract glucose chloramphenicol) agar is added. When the agar is solidified, plates are incubated at 35°C for 48 hours before colony counting [6]. The performance characteristics of the EN 15789 method reported after logarithmic transformation (CFU) are:

- a repeatability standard deviation (s_r) ranging from 0.17 to 0.36 $\log_{10}$ CFU/g,
- a reproducibility standard deviation (s_R) ranging from 0.55 to 0.60 $\log_{10}$ CFU/g, and,
- a limit of detection (LOD) of $1x10^5$ CFU/kg, well below the minimum dose proposed by the applicant.

Based on these performance characteristics the EURL recommends, for official control, the CEN method EN 15789 for the enumeration of *Saccharomyces cerevisiae* NCYC R404 in *feed additive, premixtures* and *feedingstuffs*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends Polymerase Chain Reaction (PCR) method for the identification of the yeast strain and the CEN method (EN 15789) for the enumeration of *Saccharomyces cerevisiae* NCYC R404 in *feed additive*, *premixtures* and *feedingstuffs.*

Recommended text for the register entry (analytical method)

- Identification: Polymerase Chain Reaction (PCR)
- Enumeration: Pour plate method using yeast extract dextrose chloramphenicol (CGYE) agar - EN 15789

5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *MycoCell* have been sent to the European Union Reference Laboratory for Feed Additives. The dossier has been made available to the EURL by EFSA.

6. REFERENCES

[1] *Application/Ref:SANCO/1831/0060-2012

[2] *Application, Proposal for Register Entry, Annex A

[3] *Technical dossier, Section II: Identity, characterisation and conditions of use of the additive; methods of analysis

[4] *Technical dossier, Section II, 2.2 Characterisation of the active substance(s)/agent(s)

[5] ISO 7218:2007 'Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations'

[6] EN 15789:2009 'Animal feeding stuffs - Isolation and enumeration of yeast probiotic strains'

*Refers to Dossier no: FAD-2012-0038

7. RAPPORTEUR LABORATORY

The Rapporteur Laboratory for this evaluation was the European Union Reference Laboratory for Feed Additives, IRMM, Geel, Belgium. This report is in accordance with the opinion of the consortium of National Reference Laboratories as referred to in Article 6(2) of Commission Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009.

8. ACKNOWLEDGEMENTS

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