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

Dossier related to: FAD-2008-0058
CRL/080032

Name of Additive: *Saccharomyces cerevisiae* MUCL 39885

Active Agent(s): *Saccharomyces cerevisiae* MUCL 39885

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Date: 26/01/2010

EXECUTIVE SUMMARY

In the current application authorisation is sought for the microbial feed additive *Saccharomyces cerevisiae* MUCL 39885 under the category 'zootechnical additives', functional group 'gut flora stabilizers' according to Annex I of Regulation (EC) No 1831/2003. Specifically, authorization is sought for the use of *Saccharomyces cerevisiae* MUCL 39885 for horses. The feed additive is intended to be mixed at a minimum dose of 3×10^9 to 6×10^9 CFU/kg of *feedingstuffs*.

For the enumeration of the yeast probiotic strain *Saccharomyces cerevisiae* MUCL 39885 in *feed additive*, *premixtures* and *feedingstuffs*, the applicant proposes the ring-trial validated CEN method EN 15789:2009. This pour plate method was ring-trial validated using *feed samples* containing 10^9 to 10^{13} CFU *Saccharomyces cerevisiae*/kg. The performance characteristics of the CEN method using CGYE (yeast extract glucose chloramphenicol) agar - 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 1×10^5 CFU/kg, well below the minimum dose proposed by the applicant.

Based on these acceptable performance characteristics, the CRL recommends for official control the CEN method EN 15789:2009 for the determination of the *Saccharomyces cerevisiae* MUCL 39885 in *feed additive*, *premixtures* and *feedingstuffs*.

Molecular methods were used by the applicant for strain identification. The CRL recommends for official control PCR typing, a generally recognised standard methodology for microbial identification, for the yeast *Saccharomyces cerevisiae* strain.

Further testing or validation is not considered necessary.

KEYWORDS

Saccharomyces cerevisiae, MUCL 39885, zootechnical additives, horses, gut flora stabilizers.

1. BACKGROUND

Saccharomyces cerevisiae MUCL 39885 for horses is a feed additive for which authorisation is sought under the category of 'zootechnical additives' functional group 'gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003 [1]. The yeast strain is deposited in the Belgian Collection of Microorganisms BCCM™/ MUCL Culture Collection Mycotheque de Universite Catholique de Louvain [2]. Specifically, authorisation is sought for horses the feed additive is intended to be mixed at dosage of 3×10^9 to 6×10^9 CFU/kg of *feedingstuffs* [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 authorizations 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 dossier, the methods of analysis submitted in connection with the *Saccharomyces cerevisiae* MUCL 39885 dossier 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

Qualitative and quantitative composition of the additive

For identification and characterization of the strain *Saccharomyces cerevisiae* MUCL 39885 the applicant used PCR typing [4]. This method is suitable for the purpose of analysis.

The CRL recommends for official control PCR typing, a generally recognised standard methodology for genetic identification of yeast strains.

Qualitative and quantitative composition of any impurities in the additive

The applicant analysed the *feed additive* for microbial contaminants (such as Enterobacteria, *Escherichia coli*, *Salmonella spp.* and moulds) by using appropriate EN ISO tests [5]. For undesirable substances (i.e. lead, arsenic, mercury, cadmium, aflatoxins, ochratoxin) internationally recognised standard methods are available at the respective Community 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* MUCL 39885 in *feed additive, premixtures* and *feedingstuffs* the applicant proposes the CEN method -EN 15789:2009- an international recognized pour plate method. The sample is suspended and diluted in a buffer solution and transferred to a CGYE (yeast extract glucose chloramphenicol) agar plate. The plates are then placed in an incubator at 35°C for 2 days [6]. This method was ring-trial validated using *feed samples* containing 10^9 to 10^{13} CFU *Saccharomyces cerevisiae* per kg. The performance characteristics of the CEN method using CGYE agar - reported after logarithmic transformation (CFU) - are:

- a repeatability standard deviation (s_r) of 0.17 to 0.36 \log_{10} CFU/g,
- a reproducibility standard deviation (s_R) of 0.55 to 0.60 \log_{10} CFU/g, and
- a limit of detection (LOD) of 1×10^5 CFU/kg [7], well below the minimum dose proposed by the applicant (3×10^9 CFU/kg in *feedingstuffs*).

Based on these performance characteristics, the CRL recommends for official control the standard method EN 15789:2009, using CGYE agar for the enumeration of *Saccharomyces cerevisiae* MUCL 39885 in the *feed additives, premixtures* and *feedingstuffs*.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorization the CRL recommends the CEN method (EN 15789:2009) for the enumeration of the active agent *Saccharomyces cerevisiae* MUCL 39885.

For the analysis of the identity of the bacterial strain *Saccharomyces cerevisiae* MUCL 39885 the CRL recommends for official control PCR typing, a generally recognized standard methodology for strain identification of yeast strains.

Further testing or validation is not considered necessary.

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

- Enumeration: Pour plate method using CGYE (yeast extract glucose chloramphenicol) agar (EN 15789:2009).
- Identification: PCR (Polymerase Chain Reaction) typing.

5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

In accordance with the requirements of Regulation (EC) No 1831/2003, samples of the additive *Saccharomyces cerevisiae* MUCL 39885 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] *Application/Ref: SANCO/D/2: Forw.Appl.1831/024-2009
- [2] * Technical dossier, Section 2.2.1.2 Micro-organism
- [3] *Application, Annex A, Proposal for register entry
- [4] * Technical dossier Annex II.5.PCR
- [5] * Technical dossier 2.2.2. Relevant properties
- [6] EN 15789:2009 "Animal feeding stuffs- Isolation and enumeration of yeast probiotic strains"
- [7] ISO 7218:2007 "Microbiology of food and animal feeding stuffs- General requirements and guidance for microbiological examinations"

* Refers to Dossier no: FAD-2009-0058

7. RAPPORTEUR LABORATORY

The Rapporteur Laboratory for this evaluation was Community 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.

8. ACKNOWLEDGEMENTS

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