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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 the Application for Authorisation as a Feed Additive according to Regulation (EC) No 1831/2003

Dossier related to:	FAD-2010-0038 CRL/ 100031
Name of product:	Actisaf® Sc47
Active Agent (s):	*Saccharomyces cerevisiae* NCYC Sc47
Rapporteur Laboratory:	Community Reference Laboratory for Feed Additives (CRL-FA) Geel, Belgium
Report prepared by:	Dijana Mitic (CRL-FA)
Report checked by: Date:	Piotr Robouch (CRL-FA) 15/12/2010
Report approved by: Date:	Christoph von Holst (CRL-FA) 16/12/2010

EXECUTIVE SUMMARY

In the current application authorisation is sought under articles 4(1) and 10(2) for feed additive *Actisaf® Sc47* under the category 'zootechnical additives', functional group 4(b) 'gut flora stabilisers', according to Annex I of Regulation (EC) No 1831/2003. Specifically, the authorisation is sought for the use of *Actisaf® Sc47* for rabbits for fattening, pet rabbits and non-food producing rabbits. The *feed additive* is intended to be marketed in three different forms as Actisaf® Sc47 Powder, Actisaf® Sc47 Standard and Actisaf® Sc47 HR+. The active component of *Actisaf® Sc47* is a pure culture of the strain *Saccharomyces cerevisiae* NCYC Sc47, with a minimum concentration of $5x10^9$ CFU/g. It is intended to be mixed to complete *feedingstuffs* at a minimum dose of $5x10^9$ CFU/kg.

For the enumeration of *Saccharomyces cerevisiae* NCYC Sc47 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 ($5x10^9$ CFU/kg of *feedingstuffs*).

Based on these performance characteristics the CRL recommends, for official control, the CEN method EN 15789 for the enumeration of *Saccharomyces cerevisiae* NCYC Sc47 in *feed additives*, *premixtures* and *feedingstuffs*.

Molecular methods were used by the Applicant for identification of the active agent. The CRL recommends for official control Polymerase Chain Reaction (PCR), a generally recognised standard methodology for identification of yeasts.

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

Saccharomyces cerevisiae NCYC Sc47, zootechnical additives, gut flora stabilisers, rabbits for fattening, pet and non-food producing rabbits.

1. BACKGROUND

In the current application authorisation is sought under articles 4(1) (new use) and 10(2) (re-evaluation of an authorised additive) for feed additive *Actisaf® Sc47* under the category 'zootechnical additives', functional group 4(b) 'gut flora stabilisers', according to Annex I of Regulation (EC) No 1831/2003 [1]. The *feed additive* is already authorised under Commission Regulation (EC) No 316/2003 for cattle for fattening, (EC) No 1288/2004 for sows, (EC) No 2148/2004 for piglets, (EC) No 600/2005 for fattening rabbits, (EC) No 811/2005 for dairy cows, (EC) No 1447/2006 for lambs for fattening, (EC) No 186/2007 for horses, (EC) No 188/2007 for dairy goats and dairy sheep, (EC) No 209/2008 for pigs for fattening and (EC) No 232/2009 for dairy buffaloes. The active component of *Actisaf® Sc47* is a pure culture of the strain *Saccharomyces cerevisiae* NCYC Sc47, with a minimum concentration of $5x10^9$ CFU/g [2, 3]. The strain is deposited at the 'National Collection of Yeast Culture (NCYC)' in Norwich, United Kingdom [4]. Specifically, the authorisation is sought for the use of *Actisaf® Sc47* for rabbits for fattening, pet rabbits and non-food producing rabbits. The *feed additive* is intended to be marketed in three different forms as:

- Actisaf® Sc47 Powder,
- Actisaf® Sc47 Standard, and
- Actisaf® Sc47 HR+ [5].

It is intended to be mixed to complete *feedingstuffs* at a minimum dose of $5x10^9$ CFU/kg of *Saccharomyces cerevisiae* NCYC Sc47 [2].

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 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 or group of applications. For this dossier, the methods of analysis submitted in connection with *Actisaf® Sc47*, 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 impurities in the additive

For identification and characterisation of the strain *Saccharomyces cerevisiae* NCYC Sc47 the Applicant used molecular methods such as Polymerase Chain Reaction (PCR) [6]. This method is suitable for the purpose of analysis. The CRL recommends for official control Polymerase Chain Reaction (PCR), a generally recognised standard methodology for identification of yeasts [7].

Qualitative and quantitative composition of any impurities in the additive

The Applicant analysed the *feed additive* for microbial contaminants (such as *Escherichia coli, Salmonella*, *Staphylococcus aureus*, coliforms and mesophilic flora) using appropriate ISO tests [8].

For undesirable substances (i.e. arsenic, cadmium, mercury, lead, selenium, copper, zinc, chrome, aflatoxins) 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 the active substance in feed additive, premixtures, feedingstuffs and water

For the enumeration of *Saccharomyces cerevisiae* NCYC Sc47 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) [9]. The sample is suspended in phosphate buffered saline (PBS) and diluted in a peptone salt solution. The appropriate dilutions are then transferred to Petri dishes and melted CGYE agar is added. When the agar is solidified, plates are incubated at 35°C for 48 hours before colony counting. The performance characteristics of the CEN 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 [10], well below the minimum dose proposed by the applicant ($5x10^9$ CFU/kg of *feedingstuffs*).

Based on these performance characteristics the CRL recommends, for official control, the CEN method EN 15789 for the enumeration of *Saccharomyces cerevisiae* NCYC Sc47 in *feed additives*, *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 CRL recommends the CEN method - EN 15789 - for the enumeration of the active agent *Saccharomyces cerevisiae* NCYC Sc47 in *feed additive*, *premixtures* and *feedingstuffs*.

For the identification of the yeast strain *Saccharomyces cerevisiae* NCYC Sc47 the CRL recommends Polymerase Chain Reaction (PCR) for official control.

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

Recommended text for the register entry (analytical method)

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

5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Actisaf® Sc47* 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] *Application/Ref:SANCO/D/2:Forw.Appl.1831/0028-2010.
[2] *Application, Proposal for Register Entry, Annex A
[3] *Technical dossier, Section II/2.1.3. Qualitative and quantitative composition of the additive
[4] *Technical dossier, Section II, Annex_II_2_a_Certificate of deposition of the strain
[5] *Technical dossier, Section II, 2.1.5. Physical state of each form of the product
[6] *Technical dossier, Annex_II_6_c_Validation of the methods for identification
[7] European Community Project SMT4-CT98-2235.'Methods for the Official Control of Probiotics Used as Feed Additives, Volume 1. 2002. Report 20873-1. Office for official Publications of the European Communities. ISBN 92-894-6250-7 (Vol. I)
[8] *Technical dossier, Section II, 2.6.3. Methods of analysis relating to the identity and characterization of the additive
[9] EN 15789:2009 'Animal feeding stuffs - Isolation and enumeration of yeast probiotic strains'
[10] ISO 7218:2007 'Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations'
*Refers to Dossier no: FAD-2010-0038

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

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, as last amended by Regulation (EC) No 885/2009.

8. ACKNOWLEDGEMENTS

The following National Reference Laboratories contributed to this report:

- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes (FR)
- Państwowy Instytut Weterynaryjny, Puławy (PL)
- Sächsische Landesanstalt für Landwirtschaft, Fachbereich 8 — Landwirtschaftliches Untersuchungswesen, Leipzig (DE)