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**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**

Actisaf[®] Sc 47
(FAD-2010-0264; CRL/100192)



**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**

Dossier related to: **FAD-2010-0264 – CRL/100192**

Name of Product: **Actisaf® Sc 47**

Active Agent (s): ***Saccharomyces cerevisiae* NCYC Sc 47**

Rapporteur Laboratory: **Centre wallon de Recherches
agronomiques (CRA-W), Gembloux,
Belgium**

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Date: **(EURL-FA)
07/07/2016**

Report approved by: **Christoph von Holst**
Date: **07/07/2016**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 10 (2) for *Actisaf® Sc 47* under the category / functional group 4(b) 'zootechnical additives' / 'gut flora stabilisers', according to Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for the use of the *feed additive* for weaned piglets, sows, dairy cows and cattle for fattening.

According to the Applicant, the *feed additive* contains as *active substance* viable cells of the non-genetically modified strain *Saccharomyces cerevisiae* NCYC Sc 47. The *feed additive* is marketed in powder form containing a minimum *Saccharomyces cerevisiae* NCYC Sc 47 concentration of 5×10^9 Colony Forming Unit (CFU)/g. The *feed additive* is to be used directly in *feedingstuffs* or through *premixtures* at minimum doses ranging from 4×10^8 to 5×10^9 CFU/kg complete *feedingstuffs*, depending on the animal species of concern.

For the identification of *Saccharomyces cerevisiae* NCYC Sc 47, the EURL recommends for official control Polymerase Chain Reaction (PCR), a generally recognised standard methodology for genetic identification of yeasts.

For the enumeration of *Saccharomyces cerevisiae* NCYC Sc 47 in *feed additive*, *premixtures* and *feedingstuffs*, the Applicant submitted the ring-trial validated pour plate method EN 15789. Based on the performance characteristics available, the EURL recommends this method 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

Saccharomyces cerevisiae NCYC Sc 47, zootechnical additives, gut flora stabilisers, weaned piglets, sows, dairy cows, cattle for fattening.

1. BACKGROUND

In the current application authorisation is sought under Article 10 (2) (re-evaluation of an authorised additive) for *Actisaf® Sc 47* under the category / functional group 4(b) 'zootechnical additives' / 'gut flora stabilisers', according to Annex I of Regulation (EC) No 1831/2003 [1]. Authorisation is sought for the use of the *feed additive* for weaned piglets, sows, dairy cows and cattle for fattening.

According to the Applicant, the *feed additive* contains as *active substance* viable cells of the non-genetically modified strain *Saccharomyces cerevisiae* NCYC Sc 47 [2]. The strain is deposited at the National Collection of Yeast Culture (NCYC) (Norwich, UK) [3].

The *feed additive* is intended to be marketed in three different forms of powder containing a minimum *Saccharomyces cerevisiae* NCYC Sc 47 concentration of 5×10^9 Colony Forming Unit (CFU)/g [4].

The *feed additive* is to be used directly in *feedingstuffs* or through *premixtures* at minimum doses ranging from 4×10^8 to 5×10^9 CFU/kg complete *feedingstuffs*, depending on the animal species of concern [5,6].

Note: The feed additive is already authorised under the following Commission Regulations (*): No 316/2003 (cattle for fattening); No 1288/2004 (sows); No 2148/2004 (weaned piglets); No 1811/2005 (dairy cows); No 1447/2006 (lambs for fattening); No 186/2007 (horses); No 188/2007 (dairy goats and dairy sheep); No 209/2008 (pigs for fattening); No 232/2009 (dairy buffaloes); and under Commission Regulations No 600/2005 (rabbits for fattening) and No 883/2010 (calves for rearing).

(*) last amended by Commission Implementing Regulation No 1018/2012.

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 for each application or group of applications. For this particular dossier, the methods of analysis submitted in connection with *Actisaf® Sc 47* 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

For the identification and characterisation of the strain *Saccharomyces cerevisiae* NCYC Sc 47, the Applicant used molecular methods including Polymerase Chain Reaction (PCR) [3]. The EURL recommends for official control Polymerase Chain Reaction (PCR), a generally recognised standard methodology for genetic identification of yeasts [7].

Qualitative and quantitative composition of impurities in the additive

The Applicant analysed the *feed additive* for microbial contaminants (e.g. *Escherichia coli*, coliforms, *Staphylococcus aureus*, *Salmonella* and mesophilic flora) using the methods mentioned in the technical dossier [8]. As for the determination of other undesirable substances in the *feed additive* (e.g. arsenic, cadmium, lead, mercury, mycotoxins), analytical methods for official control are available from the respective European Union Reference Laboratories [9].

Description of the analytical methods for the determination of the active substances in feed additive, premixtures and feedingstuffs

For the enumeration of *Saccharomyces cerevisiae* NCYC Sc 47 in *feed additive*, *premixtures* and *feedingstuffs*, the Applicant submitted the ring-trial validated pour plate method EN 15789 developed by CEN [8].

The sample is suspended in phosphate buffered saline and diluted in peptone salt solution; the appropriate dilutions are then transferred to Petri dishes and melted CGYE (yeast extract dextrose chloramphenicol agar) is added. When the agar is solidified, plates are incubated at 35 °C for 48 hours before colony counting. The following performance characteristics were reported after logarithmic transformation (CFU) [10]:

- a standard deviation for repeatability (S_r) ranging from 0.17 to 0.36 \log_{10} CFU/g;
- a standard deviation for reproducibility (S_R) ranging from 0.55 to 0.60 \log_{10} CFU/g; and
- a limit of quantification (LOQ) of 3×10^5 CFU/kg [11].

Based on the performance characteristics presented, the EURL recommends for official control the ring-trial validated EN 15789 method for the enumeration of *Saccharomyces cerevisiae* NCYC Sc 47 in *feed additive*, *premixtures* and *feedingstuffs*.

Note: The EN 15789 method is not applicable to mineral feeds composed mainly of minerals and containing at least 40% crude ash. For these matrices laboratories may consider using the ring-trial validated VDLUFA method 28.2.6 instead [12].

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 for official control Polymerase Chain Reaction (PCR) for the identification of *Saccharomyces cerevisiae* NCYC Sc 47 and the ring-trial validated pour plate method EN 15789 for enumeration of this strain in *feed additive, premixtures and feedingstuffs*.

Recommended text for the register entry (analytical method)

- Identification: Polymerase Chain Reaction (PCR)
- Enumeration in *feed additive, premixtures and feedingstuffs*: Pour plate method using yeast extract dextrose chloramphenicol agar (CGYE) (EN 15789)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Actisaf® Sc 47* 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, Reference SANTE G1: F.A. 1831/0022-2015
- [2] *Technical dossier, Section II/2.2. Characterisation of the active substance
- [3] *Technical dossier, Section II, Annex_II_2_a Certificate of deposition of the strain
- [4] *Technical dossier, Section II/2.1. Identity of the additive
- [5] *Technical dossier, Section II/2.5. Conditions of use of the additive
- [6] *Application, Proposal for Register Entry, Annex A
- [7] European Community Project SMT4-CT98-2235."Methods for the Official Control of Probiotics Used as Feed Additives", Report 20873/1 EN (2002) ISBN 92-894-6250-7 (Vol. I) and Report 20873/3 EN (2002) ISBN 92-894-6252-3 (Vol. III)
- [8] *Technical dossier, Section II/2.6. Methods of analysis and reference samples
- [9] Commission Regulation (EC) No 776/2006 amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards to Community Reference Laboratories
- [10] EN 15789:2009 - Animal feeding stuffs - Isolation and enumeration of yeast probiotic strains

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- [11] EN ISO 7218:2007 - Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations
- [12] VDLUFA method–Enumeration of *Saccharomyces cerevisiae* (VDLUFA Methodenbuch Bd.III, 28.2.6)

*Refers to Dossier no: FAD-2010-0264

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was Centre wallon de Recherches agronomiques (CRA-W), Gembloux, 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 (EU) No 2015/1761.

8. ACKNOWLEDGEMENTS

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- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, PESCA, Alimentació i Medi Natural. Generalitat de Catalunya, Cabrils (ES)
- Laboratoire de Rennes (SCL L35), Service Commun des Laboratoires DGCCRF et DGDDI, Rennes (FR)
- Univerza v Ljubljani. Veterinarska fakulteta. Nacionalni veterinarski inštitut. Enota za patologijo prehrane in higieno okolja, Ljubljana (SI)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Państwowy Instytut Weterynaryjny, Pulawy (PL)