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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<sup>®</sup> Sc 47**  
*(FAD-2015-0027; CRL/150006)*





**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-2015-0027 – CRL/150006**

Name of Product: **Actisaf® Sc 47**

Active Agent (s): ***Saccharomyces cerevisiae* NCYC Sc 47 /  
CNCM I-4407**

Rapporteur Laboratory: **Centro di referenza nazionale per la  
sorveglianza ed il controllo degli alimenti  
per gli animali (CReAA), Torino, Italy**

Report prepared by: **Alessandro Benedetto**

Report checked by: **Stefano Bellorini**  
Date: **& Piotr Robouch (EURL-FA)  
11/07/2016**

Report approved by: **Christoph von Holst**  
Date: **11/07/2016**

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## EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 10(2) for *Actisaf® Sc47* 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 fattening lambs.

According to the Applicant, the *feed additive* contains as active substance viable cells of the non-genetically modified strain *Saccaromyces cerevisiae* NCYC Sc 47 / CNCM I-4407. The *feed additive* is intended to be marketed in powder form, containing a minimum *Saccaromyces cerevisiae* NCYC Sc 47 / CNCM I-4407 concentration of  $5 \times 10^9$  Colony Forming Unit (CFU)/g. The *feed additive* is to be used directly in *feedingstuffs* or through *premixtures* at minimum dose of  $1.4 \times 10^9$  CFU/kg complete *feedingstuffs*.

For the identification of *Saccaromyces cerevisiae* NCYC Sc 47 / CNCM I-4407, the EURL recommends for official control Polymerase Chain Reaction (PCR), a recognised standard methodology for genetic identification of yeast.

For the enumeration of *Saccaromyces cerevisiae* NCYC Sc 47 / CNCM I-4407 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

*Saccaromyces cerevisiae* NCYC Sc 47 / CNCM I-4407, zootechnical additives, gut flora stabilizers, fattening lambs.

## 1. BACKGROUND

In the current application authorisation is sought under Article 10(2) (re-evaluation of the authorised *feed 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 *Actisaf*® *Sc 47* for lambs 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 / CNCM I-4407 [2,3]. The strain is deposited at the "European culture collection CNCM Institut Pasteur", France [4].

The *feed additive* is intended to be marketed in three different forms of powder with a minimum *Saccharomyces cerevisiae* NCYC Sc 47 / CNCM I-4407 concentration of  $5 \times 10^9$  Colony Forming Unit (CFU)/g [5].

The *feed additive* is intended be used directly in *feedingstuffs* or through *premixtures* at minimum dose of  $1.4 \times 10^9$  CFU/kg complete *feedingstuffs* [3].

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 (EU) 2015/1761, 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 strain *Saccharomyces cerevisiae* NCYC Sc 47 / CNCM I-4407 the Applicant used molecular methods, including Polymerase Chain Reaction

(PCR) [6,7]. The PCR method was validated for identification of several authorised probiotic yeast strains in animal feed, including *Saccaromyces cerevisiae* NCYC Sc 47 / CNCM I-4407. 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 impurities (*Escherichia coli*, *Salmonella spp*, *Staphylococcus aureus*, total coliforms and mesophilic flora) using the ISO 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, aflatoxin B1 and dioxins), 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 *Saccaromyces cerevisiae* NCYC Sc 47 / CNCM I-4407 in *feed additive, premixtures* and *feedingstuffs* the Applicant submitted the ring-trial validated pour plate method for the enumeration of yeast probiotic strains (EN 15789) [10,11].

The sample is suspended in phosphate buffered saline (PBS) and diluted in a peptone salt solution. The appropriate dilutions are then mixed on Petri plates with melted CYGE agar. Once solidified, plates are incubated at 35 °C for 48 hours before colony counting. The following performance characteristics were reported after logarithmic transformation (CFU):

- a standard deviation for repeatability ( $S_r$ ) of 0.17-0.36  $\log_{10}$  CFU/g;
- a standard deviation for reproducibility ( $S_R$ ) of 0.55-0.60  $\log_{10}$  CFU/g; and
- a limit of quantification (LOQ) of  $3 \times 10^5$  CFU/kg [12].

Based on the performance characteristics presented, the EURL recommends for official control the ring-trial validated EN 15789 method for the enumeration of *Saccaromyces cerevisiae* NCYC Sc 47 / CNCM I-4407 in *feed additive, premixtures* and *feedingstuffs*.

Note: The EN 15789 method is not applicable to mineral feeds containing more than 40% crude ash. For these matrices laboratories may consider the validated 28.2.6.VDLUFA method [13]

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 / CNCM I-4407 and the ring-trial validated pour plate method EN 15789 for the enumeration of the strain *Saccharomyces cerevisiae* NCYC Sc 47 / CNCM I-4407 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 SANCO/G1: Forw. Appl. 1831/0026-2015
- [2] \*Application, Proposal for Register Entry – Annex A
- [3] \*Technical dossier, Section II: 2.5 Condition of the use of the additive
- [4] \*Technical dossier, Section II: 2.2 Characterisation of the active substance
- [5] \*Technical dossier, Section II: 2.1.5 Physical state of each form of the product
- [6] \*Technical dossier, Section II: 2.6.3 Methods of analysis relating to the identity and characterization of the additive
- [7] \*Appendix II-6-c: Validation of the official control methods based on polymerase chain reaction (PCR) for identification of authorised probiotic yeast in animal feedingstuffs.
- [8] \*Technical dossier, Section II: 2.6.3 Methods of analysis relating to the identity and characterization of the additive: Purity (2.1.4)
- [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] \*Technical dossier, Section II: 2.6.1 Protocol of the methods of analysis for the active substance
- [11] EN 15789:2009 ‘Animal feeding stuffs – Isolation and enumeration of yeast probiotic strains’
- [12] ISO 7218:2007 ‘Microbiology of food and animal feedingstuffs – General requirements and guidance for microbiological examinations’
- [13] VDLUFA method ‘Enumeration of *Saccharomyces cerevisiae* (VDLUFA Methodenbuch Bd.III, 28.2.6)

\*Refers to Dossier no: FAD-2015-0027

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## 7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was "Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino, Italy". 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) 2015/1761.

## 8. ACKNOWLEDGEMENTS

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