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EURL 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-2010-0051 EURL/ 100043
Name of product:	inteSwine
Active Agent (s):	*Saccharomyces cerevisiae* NCYC R-625
Rapporteur Laboratory:	European Union Reference Laboratory for Feed Additives (EURL-FA) Geel, Belgium
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EXECUTIVE SUMMARY

In the current application authorisation is sought under article 4(1) for product *inteSwine* under the category 'zootechnical additives', functional group 4(b) 'gut flora stabilisers', according to Annex I of Regulation (EC) No 1831/2003. The active component of *inteSwine* is a pure culture of the strain *Saccharomyces cerevisiae* NCYC R-625, with a minimum concentration of $9x10^{10}$ Colony Forming Units (CFU)/g. Specifically, the authorisation is sought for the use of *inteSwine* for weaned piglets. The *feed additive* is intended to be mixed to complete *feedingstuffs* at a dose ranging from $6x10^{9}$ to $4.5x10^{10}$ CFU/kg of *Saccharomyces cerevisiae* NCYC R-625.

For the enumeration of *Saccharomyces cerevisiae* NCYC R-625 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 ($6x10^{9}$ CFU/kg of *feedingstuffs*).

Based on these performance characteristics the EURL recommends, for official control, the CEN method EN 15789 for the enumeration of *Saccharomyces cerevisiae* NCYC R-625 in *feed additives*, *premixtures* and *feedingstuffs*.

Molecular methods were used by the Applicant for identification of the active agent. The EURL 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 R-625, zootechnical additives, gut flora stabilisers, weaned piglets.

1. BACKGROUND

In the current application authorisation is sought under article 4(1) for product *inteSwine* 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 *inteSwine* is a pure culture of the strain *Saccharomyces cerevisiae* NCYC R-625, with a minimum concentration of $9x10^{10}$ 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 *inteSwine* for weaned piglets. The *feed additive* is intended to be mixed to complete *feedingstuffs* at a dose ranging from $6x10^{9}$ to $4.5x10^{10}$ CFU/kg of *Saccharomyces cerevisiae* NCYC R-625 [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 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 *inteSwine*, 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 R-625 the Applicant used molecular methods such as Chromosome Fingerprinting technique [4]. This method is suitable for the purpose of analysis. However, the EURL recommends for official control Polymerase Chain Reaction (PCR), a generally recognised standard methodology for identification of yeasts [5].

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*, *Clostridium perfringes*, coliforms and mesophilic flora) using appropriate AOAC tests [6].

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

For the enumeration of *Saccharomyces cerevisiae* R-625 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) [7]. 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 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 [8], well below the minimum dose proposed by the applicant ($6x10^9$ CFU/kg of *feedingstuffs*).

Based on these performance characteristics the EURL recommends, for official control, the CEN method EN 15789 for the enumeration of *Saccharomyces cerevisiae* R-625 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 EURL recommends the CEN method - EN 15789 - for the enumeration of the active agent *Saccharomyces cerevisiae* NCYC R-625 in *feed additive*, *premixtures* and *feedingstuffs*.

For the identification of the yeast strain *Saccharomyces cerevisiae* NCYC R-625 the EURL recommends Polymerase Chain Reaction (PCR) for official control.

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 *inteSwine* 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/D/2:Forw.Appl.1831/0031-2010.
[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] 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)
[6] *Technical dossier, Section II, 2.6.3 Methods of analysis relating to the identity and characteristion of the additive
[7] EN 15789:2009 'Animal feeding stuffs - Isolation and enumeration of yeast probiotic strains'
[8] ISO 7218:2007 'Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations'

*Refers to Dossier no: FAD-2010-0051

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was 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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- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Sächsische Landesanstalt für Landwirtschaft, Fachbereich 8 — Landwirtschaftliches Untersuchungswesen, Leipzig (DE)