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**European Union Reference Laboratory for Feed Additives**

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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

## Saccharomyces cerevisiae CNCM I-1079

***(FAD-2010-0121; CRL/100166)***

European Union Reference Laboratory
Feed Additives

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| Dossier related to: | **FAD-2010-0121 - CRL/100166** |
| Name of Feed Additive: | **Levucell SB 20/Levucell SB10 ME/Levucell SB 10 ME Titan** |
| Active Agent (s): | **Saccharomyces cerevisiae CNCM I-1079** |
| Rapporteur Laboratory: | **National Veterinary Research Institute (NVRI)** |
| Report prepared by: | **Marek Walczak, Krysztof Kwiatek** |
| Report checked by:<br>Date: | **Rebeca Fernández Orozco, Zigmas Ezerskis and Piotr Robouch (EURL-FA)**<br>**08/01/2015** |
| Report approved by:<br>Date: | **Christoph von Holst**<br>**09/01/2015** |

## EXECUTIVE SUMMARY

In the current application authorisation is sought under article 10(2) for *Saccharomyces cerevisiae* CNCM I-1079 under the category / functional group 4(a) "zootechnical additives' / 'digestability enhancers' and 'gut flora stabilisers', according to Annex I of Regulation (EC) No 1831/2003.

The *feed additive* is intended to be marketed as a powder (Levucell SB 20) or in micro-encapsulated form (Levucell SB 10 ME and Levucell SB 10 ME Titan). The active substance of the *feed additive* are viable cells of *Saccharomyces cerevisiae* CNCM I-1079 at a minimum concentration of $1x10^{10}$ CFU/g product. The strain is deposited at Collection Nationale de Cultures de Micro-organismes (C.N.C.M.) at Pasteur Institute.

The *feed additive* is intended to be used in *feedingstuffs* through *premixtures*. Specifically, the authorisation is sought for the use of the *feed additive* for sows and piglets (weaned) at a minimum dose of $1x10^9$ and $2x10^9$ CFU / kg *feedingstuffs*, respectively.

For the identification of *Saccharomyces cerevisiae* CNCM I-1079 the Applicant submitted the polymerase chain reaction (PCR) amplification method, a generally recognised methodology for microbial identification. This method was ring-trial validated to become the CEN technical standard (CEN/TS 15790:2008). The latter is recommended by EURL for official control to identify the *Saccharomyces cerevisiae* CNCM I-1079.

For the enumeration of *Saccharomyces cerevisiae* CNCM I-1079 in *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted 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 log10 CFU/g; - a reproducibility standard deviation ($s_R$) ranging from 0.55 to 0.60 log10 CFU/g; and - a limit of quantification (LOQ) of $1x10^5$ CFU/kg, well below the minimum dose proposed by the Applicant.

Based on these performance characteristics the EURL recommends for official control, the CEN method EN 15789 for the enumeration of *Saccharomyces cerevisiae* CNCM I-1079 in *feed additive*, *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.

**KEYWORDS**

*Saccharomyces cerevisiae* CNCM I-1079, zootechnical additives, digestibility enhancers, gut flora stabilisers, sows and piglets (weaned)

## 1. BACKGROUND

In the current application authorisation is sought under article 10(2) for *Saccharomyces cerevisiae* CNCM I-1079 under the category / functional group 4(a, b) "zootechnical additives' / 'digestability enhancers', 'gut flora stabilisers', according to Annex I of Regulation (EC) No 1831/2003 [1,2].

The *feed additive* is intended to be marketed as a powder (Levucell SB 20) or in micro-encapsulated form (Levucell SB 10 ME and Levucell SB 10 ME Titan micro-capsules, which have rod and oval shape, respectively) [3]. The active substance of the *feed additive* are viable cells of *Saccharomyces cerevisiae* CNCM I-1079 at a minimum concentration of $1x10^{10}$ Colony Forming Unit (CFU) / g product [2]. The strain is deposited at Collection Nationale de Cultures de Micro-organismes (C.N.C.M.) at Pasteur Institute [3].

The *feed additive* is intended to be used in *feedingstuffs* through *premixtures* [3]. Specifically, the authorisation is sought for the use of the *feed additive* for sows and piglets (weaned) at a minimum dose of $1x10^9$ and $2x10^9$ CFU / kg *feedingstuffs*, respectively [2].

Note: the EURL already evaluated the methods of analysis for *Saccharomyces cerevisiae* in the reports: FAD-2005-0018, FAD-2005-0016, FAD-2007-0029.

## 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 *Saccharomyces cerevisiae* CNCM I-1079 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 of *Saccharomyces cerevisiae* CNCM I-1079, the Applicant submitted the polymerase chain reaction (PCR) amplification method, a generally recognised methodology for microbial identification [4]. This method was ring-trial validated to become the CEN technical standard CEN/TS 15790:2008 [5]. The latter method is recommended by the EURL for official control to identify the *Saccharomyces cerevisiae* CNCM I-1079.

### *Quantitative composition of impurities in the additive*

The Applicant analysed the *feed additive* for microbial contaminants (such as coliforms, *Escherichia coli*, *Salmonella*, mesophilic aerobic bacteria, wild yeasts by using appropriate EN ISO standards [3]. As for the determination of other undesirable substances in the *feed additive* (e.g. arsenic, cadmium, lead, mercury, mycotoxins and dioxins) several internationally recognised standard methods are available at the respective European Union Reference Laboratories [6].

### *Description of the analytical methods for the determination of the active substance in feed additive, premixtures and feedingstuffs*

For the enumeration of *Saccharomyces cerevisiae* CNCM I-1079 in *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted the ring-trial validated CEN pour plate method for the enumeration of yeast probiotic strains (EN 15789) [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 a melted CGYE (yeast extract glucose chloramphenicol) agar is added. When the agar is solidified, plates are incubated at 35°C for 48 hours before colony counting [7,8]. 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 quantification (LOQ) of $1x10^5$ CFU/kg for *feedingstuffs*, far below the minimum dose proposed by the Applicant [8].

Based on these performance characteristics the EURL recommends for official control, the CEN method EN 15789 for the enumeration of *Saccharomyces cerevisiae* CNCM I-1079 in *feed additive*, *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 Polymerase Chain Reaction (PCR) method (CEN/TS 15790:2008) for the identification of the yeast strain and the CEN method (EN 15789) for the enumeration of *Saccharomyces cerevisiae* CNCM I-1079 in *feed additive*, *premixtures* and *feedingstuffs*.

***Recommended text for the register entry (analytical method)***

- Identification: Polymerase Chain Reaction (PCR) - CEN/TS 15790:2008
- Enumeration: Pour plate method using yeast extract dextrose chloramphenicol (CGYE) agar - EN 15789

## 5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Saccharomyces cerevisiae* CNCM I-1079 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. 1831/0008-2014

[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] Leuschner R.G.K., Bew J., Fourcassier P., Bertin G. 2004. Validation of the Official Control Methods based on polymerase chain reaction (PCR) for identification of authorised probiotic yeast in animal feedingstuffs. System. Appl. Microbiol. 27, 492-500

[5] CEN/TS 15790:2008 – PCR typing of probiotic strains of *Saccharomyces cerevisiae* (yeast)

[6] 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

[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-2010-0121

## 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

The following National Reference Laboratories contributed to this report:

- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Sachgebiet Futtermittel des Bayrischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim (DE)[1]
- Centre wallon de Recherches agronomiques (CRA-W), Gembloux (BE)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires (FR)
- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen, Jena (DE)

[1] Name and address according to Regulation (EC) No 885/2009: Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim