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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 DSM34246

(FEED-2021-1473; CRL/220006)

European Union Reference Laboratory
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Dossier related to:	**FEED-2021-1473 - CRL/220006**
Name of Product:	***Saccharomyces cerevisiae DSM34246***
Active Agent (s):	**Saccharomyces cerevisiae**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **JRC Geel, Belgium**
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Report checked by: Date:	**María José González de la Huebra** **17/04/2023**
Report approved by: Date:	**Christoph von Holst** **17/04/2023**

EXECUTIVE SUMMARY

In the current application an authorisation is sought under Article 4 for *Saccharomyces cerevisiae* DSM34246 under the category / functional group 4(b) "zootechnical additives" / "gut flora stabilisers", according to Annex I of Regulation (EC) No 1831/2003. The authorisation is sought for the use of the *feed additive* for pets and other non-food producing animals (cats and dogs).

According to the Applicant, the *feed additive* contains as *active substance* viable cells of a not genetically modified *Saccharomyces cerevisiae* DSM34246 with a minimum content of 2×10^{10} Colony Forming Unit (CFU) / g product. The *feed additive* is proposed to be directly included in the *compound feed* with a minimum level of inclusion corresponding to 5×10^{9} CFU / kg.

For the enumeration of *Saccharomyces cerevisiae* DSM34246 in the *feed additive*, and *compound feed* the Applicant proposed the recently revised ring-trial validated EN 15789 method dedicated for the enumeration of *Saccharomyces cerevisiae* in *feed additives*, *premixtures* and *compound feed* (with exclusion of mineral feed) containing *Saccharomyces cerevisiae* as a single microorganism or in a mixture with other microorganisms.

The following performance characteristics are reported in the frame of the ring-trial validation studies when using pour plate method with yeast extract dextrose chloramphenicol (YGC) agar after logarithmic transformation of CFU values ranging from 7.13 to 7.48 $\log_{10}$ CFU / g: a standard deviation for repeatability (S_r) ranging from 0.17 to 0.36 $\log_{10}$ CFU / g and a standard deviation for reproducibility (S_R) ranging from 0.55 to 0.60 $\log_{10}$ CFU / g. In addition, a limit of quantification (LOQ) of 3×10^{5} CFU / kg can be derived using the considerations of EN ISO 7218 standard.

Based on the performance characteristics available, the EURL recommends for official control the ring-trial validated EN 15789 method for the enumeration of *Saccharomyces cerevisiae* DSM34246 in the *feed additive* and *compound feed*.

For the identification of *Saccharomyces cerevisiae* DSM34246, the Applicant applied whole genome sequencing (WGS). However, the EURL recommends for official control the polymerase chain reaction (PCR) amplification method, a generally recognised methodology for microbial identification. This method was ring-trial validated and became the CEN technical specification CEN/TS 15790.

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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

KEYWORDS

Saccharomyces cerevisiae Canobios-BL DSM34246, *Saccharomyces cerevisiae*, zootechnical additives"/"gut flora stabilisers", pets and other non-food producing animals (cats and dogs).

1. BACKGROUND

In the current application an authorisation is sought under Article 4(1) (new feed additive) for *Saccharomyces cerevisiae* DSM34246 under the category / functional group 4(b) "zootechnical additives" / "gut flora stabilisers", according to Annex I of Regulation (EC) No 1831/2003 [1]. The authorisation is sought for the use of the *feed additive* for pets and other non-food producing animals (cats and dogs) [1,2].

According to the Applicant, the *feed additive* contains as *active substance* viable cells of a not genetically modified *Saccharomyces cerevisiae* with a minimum content of 2×10^{10} Colony Forming Unit (CFU) / g product [3]. The specific strain is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) with deposition code "DSM34246" [4].

The *feed additive* is intended to be marketed in small granules and mixed with lecithin with trade name *Saccharomyces cerevisiae Canobios-BL* DSM34246 [5]. The *feed additive* is proposed to be directly included in the *compound feed* with a minimum level of inclusion corresponding to 5×10^{9} CFU / kg [2]. The possible inclusion in *water* and *premixture* is not foreseen [2].

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 *Saccharomyces cerevisiae* DSM34246 and their suitability to be used for official controls in the frame of the authorisation were evaluated.

3. EVALUATION

Description of the analytical methods for the determination of the active substance in the feed additive, premixtures, feedingstuffs and when appropriate water (section 2.6.1 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

For the enumeration of *Saccharomyces cerevisiae* DSM34246 in the *feed additive*, and *compound feed* the Applicant proposed the recently revised ring-trial validated EN 15789 method dedicated for the enumeration of *Saccharomyces cerevisiae* in *feed additives*, *premixtures* and *compound feed* (with exclusion of mineral feed) containing *Saccharomyces cerevisiae* as a single microorganism or in a mixture with other microorganisms [6,7].

The sample is suspended in phosphate buffered saline containing Polysorbate 80 (Tween® 80) (tPBS). For serial dilutions, the tPBS or alternatively a peptone salt solution (PSS) can be used. The appropriate dilutions are then mixed on Petri plates with yeast extract dextrose chloramphenicol (YGC) agar using pour plate (or spread plate) methods. Alternatively, chloramphenicol can be replaced by oxytetracycline at a final concentration of 100 µg / ml of the medium and any other medium leading to comparable results can be used (e.g. Sabouraud dextrose agar (SDA) or Wort agar supplemented with chloramphenicol). The plates are incubated at 30 °C ± 1 °C for 48 to 72 h before colony counting [7].

The number of colony forming units (CFU) per g (or per ml) of feed is calculated according to the recommendations of the EN ISO 7218 standard using an equation specified in the EN 15789 standard method [7,8].

The following performance characteristics are reported in the frame of the ring-trial validation studies when using pour plate method with YGC agar after logarithmic transformation of CFU values ranging from 7.13 to 7.48 $\log_{10}$ CFU / g: a standard deviation for repeatability (S_r) ranging from 0.17 to 0.36 $\log_{10}$ CFU / g and a standard deviation for reproducibility (S_R) ranging from 0.55 to 0.60 $\log_{10}$ CFU / g [7].

In addition, a limit of quantification (LOQ) of 3 x 10^5 CFU / kg can be derived using the considerations of EN ISO 7218 standard [8].

Based on the performance characteristics available, the EURL recommends for official control the ring-trial validated EN 15789 method for the enumeration of *Saccharomyces cerevisiae* DSM34246 in the *feed additive* and *compound feed*.

Note: The EN 15789 method is not applicable to mineral feeds containing at least of 40 % (w / w) of crude ash. For these matrices laboratories may consider the validated 28.2.6.VDLUFA method [9].

Methods of analysis for the determination of the residues of the additive in food (section 2.6.2 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

An evaluation of corresponding methods of analysis is not relevant for the present application.

Identification/Characterisation of the feed additive (section 2.6.3 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

For the characterisation of *Saccharomyces cerevisiae* DSM34246, the Applicant applied whole genome sequencing (WGS) [4]. However, the EURL recommends for official control the polymerase chain reaction (PCR) amplification method, a generally recognised methodology for microbial identification [10]. This method was ring-trial validated and became the CEN technical specification CEN / TS 15790 [11].

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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends (i) Polymerase Chain Reaction (PCR) method (CEN / TS 15790) for the identification of the *Saccharomyces cerevisiae* DSM34246 strain and (ii) the CEN method (EN 15789) for the enumeration of *Saccharomyces cerevisiae* DSM34246 strain in the *feed additive* and *compound feed*.

Recommended text for the register entry (analytical method)

- Identification: Polymerase Chain Reaction (PCR) – CEN / TS 15790
- Enumeration in the *feed additive* and *compound feed*: Pour or spread plate method (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* DSM34246 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] *Forwarding of applications for authorisation of feed additives in accordance with Regulation (EC) No 1831/2003 – E-Submission Food Chain platform – https://webgate.ec.europa.eu/esfc/#/applications/2207 https://open.efsa.europa.eu/questions/EFSA-Q-2022-00373

[2] *Technical dossier, Saccharomyces Cerevisiae Canobios-BL_Gut flora stabiliser_Section1 Scientific Summary : 2.5.1. Proposed mode of use in animal nutrition

[3] *Technical dossier, Saccharomyces Cerevisiae Canobios-BL_Gut flora stabiliser_Section1 Scientific Summary : 2.1.3 Qualitative and quantitative composition

[4] *Technical dossier, Saccharomyces Cerevisiae Canobios-BL_Gut flora stabiliser_Section1 Scientific Summary : 2.2.1 Description

[5] *Technical dossier, Saccharomyces Cerevisiae Canobios-BL_Gut flora stabiliser_Section1 Scientific Summary : 2.1.3 Qualitative and quantitative composition

[6] *Technical dossier, Saccharomyces Cerevisiae Canobios-BL_Method of analysis: 2.6 Method of analysis

[7] EN 15789:2022 – Animal feeding stuffs – Detection and enumeration of Saccharomyces cerevisiae used as feed additive

[8] ISO 7218:2007 – Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations

[9] Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA). Method 28.2.6: Enumeration of Saccharomyces cerevisiae, in: Methods book Vol. III – 28.2.6

[10] 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

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

*Refers to Dossier no: FEED-2021-1473

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation is the European Union Reference Laboratory for Feed Additives, JRC, 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 (EU) 2015/1761.

8. ACKNOWLEDGEMENTS

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