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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 Y1242

(FEED-2022-6292; CRL/220040)

European Union Reference Laboratory
Feed Additives

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Dossier related to:	**FEED-2022-6292 - CRL/220040**
Name of Product:	***Saccharomyces cerevisiae Y1242***
Active Agent (s):	***Saccharomyces cerevisiae Y1242***
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **JRC Geel, Belgium**
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Report approved by: Date:	**Christoph von Holst** **22/08/2023**

EXECUTIVE SUMMARY

In the current application an authorisation is sought under Article 4 (1) for *Saccharomyces cerevisiae* Y1242 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 cattles for fattening, cows for milk production, piglets (suckling and weaned), dairy sheeps, lambs for fattening, dairy goats, goats for fattening, horses and buffalo.

The active substance of the *feed additive* is viable cells of *Saccharomyces cerevisiae* strain Y1242 with a minimum content of 2.0×10^{10} Colony Forming Unit (CFU) / g *feed additive*.

The *feed additive* is intended to be used directly in *compound feed* or through *premixtures*. The Applicant proposed the following minimum doses of the active substance / additive in *compound feed*: 3 g / animal / day (for dairy cows and other dairy ruminants); 1.5 g / animal / day (for cattle for fattening, other growing ruminants, horses and other Equidae); and 0.5 g / kg *compound feed* (for piglets and other growing Suidae).

For the identification of *Saccharomyces cerevisiae* Y1242, 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 the identification of probiotic yeast. This method was ring-trial validated and became the CEN technical specification CEN/TS 15790.

For the enumeration of *Saccharomyces cerevisiae* Y1242 in the *feed additive*, *premixtures* and *compound feed* the Applicant proposed the ring-trial validated EN 15789 method, which was recently revised by CEN resulting in updated 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 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* Y1242 in the *feed additive*, *premixtures* and *compound feed*.

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

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 Y1242, zootechnical additives, gut flora stabilisers, cattles for fattening, cows for milk production, piglets (suckling and weaned), dairy sheeps, lambs for fattening, dairy goats, goats for fattening, horses and buffalo.

1. BACKGROUND

In the current application an authorisation is sought under Article 4 (1) (new feed additive) for *Saccharomyces cerevisiae* Y1242 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 cattles for fattening, cows for milk production, piglets (suckling and weaned), dairy sheeps, lambs for fattening, dairy goats, goats for fattening, horses and buffalo [1].

The active substance of the *feed additive* is viable cells of *Saccharomyces cerevisiae* strain Y1242 with a minimum content of 2.0 x 10^{10} Colony Forming Unit (CFU) / g *feed additive* [2].

The *feed additive* is intended to be used directly in *compound feed* or through *premixtures* [3]. The Applicant proposed the following minimum doses of the active substance / additive in *compound feed*: 3 g / animal / day (for dairy cows and other dairy ruminants); 1.5 g / animal / day (for cattle for fattening, other growing ruminants, horses and other Equidae); and 0.5 g / kg *compound feed* (for piglets and other growing Suidae) [3].

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* Y1242 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* Y1242 in the *feed additive, premixtures* and *compound feed* the Applicant proposed [4] the ring-trial validated EN 15789 method, which was recently revised by CEN resulting in updated 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 [5].

According to the method, 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 as for instance Sabouraud dextrose agar (SDA) or Wort agar supplemented with chloramphenicol. The plates are incubated aerobically at 30 °C ± 1 °C for 48 to 72 h before colony counting [5].

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 [6] using an equation specified in the EN 15789 standard method [5].

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 [5].

In addition, a limit of quantification (LOQ) of $3x10^5$ CFU/kg can be derived using the considerations of EN ISO 7218 standard [6].

Furthermore, the Applicant has submitted the data from the stability and homogeneity studies of the *Saccharomyces cerevisiae* strain in the *feed additive*, *premixtures* and *compound feed* [7-10], when using a modified protocol of the EN 15789 standard method [11]. The precision values of the method derived from the log-transformed data of the mentioned studies are

comparable with the ones obtained in the frame of ring-trial validation studies of above mentioned EN 15789 standard method.

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* Y1242 in the *feed additive*, *premixtures* 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 [12].

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 identification of *Saccharomyces cerevisiae* Y1242, the Applicant applied whole genome sequencing (WGS) [13]. However, the EURL recommends for official control the polymerase chain reaction (PCR) amplification method, a generally recognised methodology for the identification of probiotic yeast [14]. This method was ring-trial validated and became the CEN technical specification CEN/TS 15790 [15].

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* Y1242 strain and (ii) the CEN method (EN 15789) for the enumeration of *Saccharomyces cerevisiae Y1242* strain in the *feed additive*, *premixtures* and *compound feed*.

Recommended text for the register entry (analytical method)

- Identification: Polymerase Chain Reaction (PCR) - CEN/TS 15790
- Enumeration in the *feed additive*, *premixtures* 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* Y1242 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/7950 https://open.efsa.europa.eu/questions/EFSA-Q-2022-00819

[2] *Technical dossier, Section II: 2.1.3. Qualitative and quantitative composition (active substance/agent, other components, impurities, batch to batch variation)

[3] *Technical dossier, Section II: 2.5. Conditions of use of the additive

[4] *Technical dossier, Section II: 2.6. Method of analysis and reference samples

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

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

[7] *Technical dossier, Section II – Annex_II.16

[8] *Technical dossier, Section II – Annex_II.17

[9] *Technical dossier, Section II – Annex_II.18

[10] *Technical dossier, Section II – Annex_II.19

[11] *Technical dossier, Section II – Annex_II.21

[12] Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA). Enumeration of Saccharomyces cerevisiae (Vol. III, method 28.2.6)

[13] *Technical dossier, Section II – Annex_II.22

[14] 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

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

*Refers to Dossier no: FEED-2022-6292

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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- Laboratoire de Rennes (SCL L35), Service Commun des Laboratoires DGCCRF et DGDDI, Rennes (FR)
- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, PESCA, Alimentació i Medi Natural. Generalitat de Catalunya, Cabrils (ES)
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