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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 DBVPG 48 SF

(FAD-2021-0040; CRL/210026)

European Union Reference Laboratory
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Dossier related to:	**FAD-2021-0040 - CRL/210026**
Name of Product:	***Saccharomyces cerevisiae DBVPG 48 SF***
Active Agent (s):	**Saccharomyces cerevisiae DBVPG 48 SF**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **JRC Geel, Belgium**
Report prepared by:	**Zigmas Ezerskis**
Report checked by: Date:	**María José González de la Huebra** **21/02/2022**
Report approved by: Date:	**Christoph von Holst** **22/02/2022**

EXECUTIVE SUMMARY

In the current application an authorisation is sought under Article 4(1) for *Saccharomyces cerevisiae* DBVPG 48SF under the category / functional group 4(a, d) 'zootechnical additives' / 'digestability enhancers', 'other zootechnical additives', according to Annex I of Regulation (EC) No 1831/2003. The authorisation is sought for the use of the *feed additive* for sows, piglets, pigs for fattening and minor growing and reproductive porcine species, dairy ruminants, ruminants for fattening, other minor growing and reproductive ruminants and horses.

The *feed additive* is intended to be marketed under 3 different forms such as powder (BioCell® P), microspheres (BioCell® S12) and micropellets (BioCell® M16). The active substance of the *feed additive* are viable cells of *Saccharomyces cerevisiae* strain DBVPG 48 SF with a minimum content of $1.0x10^9$, $1.2x10^{10}$ and $1.6x10^{10}$ Colony Forming Unit (CFU) / g products, respectively.

The *feed additive* is intended to be used in *feedingstuffs* and *premixtures*. The Applicant proposed the minimum doses of the active substance in *feedingstuffs* ranging from $4.0x10^8$ CFU/kg to $6.0x10^9$ CFU/kg, depending on the animal species.

For the identification of *Saccharomyces cerevisiae* DBVPG 48SF, the Applicant applied whole genome sequencing (WGS). However, the EURL recommends for oficial 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.

For the enumeration of *Saccharomyces cerevisiae* DBVPG 48SF in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted the ring-trial validated EN 15789 method for the enumeration of yeast probiotic strains, which was recently revised by CEN resulting in an 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 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 $3x10^5$ 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* DBVPG 48SF in *feed additive*, *premixtures* and *feedingstuffs*.

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.

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 DBVPG 48SF, zootechnical additives, digestability enhancers, other zootechnical additives, sows, piglets, pigs for fattening and minor growing and reproductive porcine species, dairy ruminants, ruminants for fattening, other minor growing and reproductive ruminants and horses.

1. BACKGROUND

In the current application an authorisation is sought under Article 4(1) (new feed additive) for *Saccharomyces cerevisiae* DBVPG 48SF under the category / functional group 4(a, d) 'zootechnical additives' / 'digestability enhancers', 'other zootechnical additives', according to Annex I of Regulation (EC) No 1831/2003 [1,2]. The authorisation is sought for the use of the *feed additive* for sows, piglets, pigs for fattening and minor growing and reproductive porcine species, dairy ruminants, ruminants for fattening, other minor growing and reproductive ruminants and horses [2].

The *feed additive* is intended to be marketed under 3 different forms such as powder (BioCell® P), microspheres (BioCell® S12) and micropellets (BioCell® M16) [3]. The active substance of the *feed additive* are viable cells of *Saccharomyces cerevisiae* strain DBVPG 48 SF with a minimum content of $1.0x10^9$, $1.2x10^{10}$ and $1.6x10^{10}$ Colony Forming Unit (CFU) / g products, respectively [4,5]. The strain is deposited in the Industrial Yeasts Collection DBVPG of the Department of Agricultural, Food and Environmental Sciences of the University of Perugia with the accession number "48 SF" [6].

The *feed additive* is intended to be used in *feedingstuffs* and *premixtures* [7,8]. The Applicant proposed the following minimum doses of the active substance in *feedingstuffs*: $4.0x10^8$ CFU/kg (for dairy cows and minor dairy species); $3.0x10^9$ CFU/kg (for horses); $4.0x10^9$ CFU/kg (for piglets and pigs for fattening and minor species; and for calves, cattle for

fattening and minor growing and fattening ruminants); and $6.0x10^9$ CFU/kg (for sows and minor species) [8].

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* DBVPG 48SF 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* DBVPG 48SF in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant proposed [9] 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 [10].

Following the updated method's protocol, 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 [10].

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 [11] using an equation specified in the EN 15789 standard method [10].

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 [10].

In addition, a limit of quantification (LOQ) of $3x10^5$ CFU/kg can be derived using the considerations of EN ISO 7218 standard [11].

In addition, the Applicant has submitted acceptable data from the stability and homogeneity studies of the *Saccharomyces cerevisiae* strain in the different forms of the *feed additive*, *premixtures* and *feedingstuffs* when using the old protocol of the EN 15789 standard method [12-15]. The EURL considers that updated protocol of the mentioned method would render equivalent data as the old one.

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* DBVPG 48SF in *feed additive*, *premixtures* and *feedingstuffs*.

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 [16].

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* DBVPG 48SF, the Applicant applied whole genome sequencing (WGS) [6]. However, the EURL recommends for official control the polymerase chain reaction (PCR) amplification method, a generally recognised methodology for microbial identification [17]. This method was ring-trial validated and became the CEN technical specification CEN/TS 15790 [18].

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* DBVPG 48SF strain and (ii) the CEN method (EN 15789) for the enumeration of *Saccharomyces cerevisiae* DBVPG 48SF strain in the *feed additive*, *premixtures* and *feedingstuffs*.

Recommended text for the register entry (analytical method)

- Identification: Polymerase Chain Reaction (PCR) - CEN/TS 15790
- Enumeration in the *feed additive*, *premixtures* and *feedingstuffs*: 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* DBVPG 48SF 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 SANTE/E5: FORW.APPL. 1831-0028-2021
[2] *Application, Annex 1 – submission number 1616078436316-2910
[3] *Technical dossier, Section II: 2.1.1. Name of the additive
[4] *Technical dossier, Section II: 2.1.3. Qualitative and quantitative composition (active substance/agent, other components, impurities, batch to batch variation)
[5] *Technical dossier, Section II: 2.3.1. Active substance(s)/agent(s)
[6] *Technical dossier, Section II: 2.2.1.2. Micro-organisms
[7] *Technical dossier, Section II: 2.4.1.2. Stability of the additive used in premixtures and feedingstuffs
[8] *Technical dossier, Section II: 2.5.1. Proposed mode of use in animal nutrition
[9] *Technical dossier, Section II: 2.6.1. Methods of analysis for the active substance
[10] EN 15789:2021 – Animal feeding stuffs – Detection and enumeration of Saccharomyces cerevisiae used as feed additive
[11] ISO 7218:2007 – Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations
[12] *Technical dossier, Section II – Annex_II.2.4.1.2
[13] *Technical dossier, Section II – Annex_II.2.4.2.2
[14] *Technical dossier, Section II – Annex_II.2.4.2.3

[15] *Technical dossier, Section II – Annex_II.2.4.2.4

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

[17] 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

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

*Refers to Dossier no: FAD-2021-0040

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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- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Instytut Zootechniki – Państwowy Instytut Badawczy, Krajowe Laboratorium Pasz, Lublin (PL)
- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, PESCA, Alimentació i Medi Natural. Generalitat de Catalunya, Cabrils (ES)