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JRC F.5/CvH/SB/AS/Ares

**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**

Pediococcus pentosaceus DSM 32292
(FAD-2021-0074; CRL/210059)



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Feed Additive according to Regulation (EC) No 1831/2003**

Dossier related to: **FAD-2021-0074 - CRL/210059**

Name of Product: ***Pediococcus pentosaceus DSM 32292***

Active Agent (s): ***Pediococcus pentosaceus DSM 32292***

Rapporteur Laboratory: **Centro di Referenza nazionale per la
sorveglianza ed il controllo degli Alimenti
per gli Animali (CReAA), Torino, Italy**

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Date: **03/04/2022**

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Date: **03/04/2022**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4 for *Pediococcus pentosaceus* DSM 32292 under the category/functional group 1(k) "technological additives"/"silage additives", according to Annex I of Regulation (EC) No 1831/2003. Specifically, the authorisation is sought for the use of the *feed additive* in *silage* for all animal species.

According to the Applicant, the *feed additive* contains as *active substance* viable cells of the strain *Pediococcus* DSM 32292. The *feed additive* is to be marketed as preparation with a minimum content of 1×10^{10} Colony Forming Units (CFU) of *Pediococcus pentosaceus* DSM 32292 /g *feed additive*. The *feed additive* is intended to be used at a minimum dose of 5×10^7 CFU/kg *silage*.

For the identification of *Pediococcus pentosaceus* DSM 32292, the EURL recommends for official control (i) Whole Genome Sequencing Analysis (WGS) or (ii) Pulsed-Field Gel Electrophoresis (PFGE). The EURL considers that both methodologies are fit for purpose for the bacterial identification of authorised additives at a strain level.

For the enumeration of *Pediococcus* DSM 32292 in the *feed additive*, the EURL recommends for official control the ring-trial validated spread plate method EN 15786.

Since the unambiguous determination of the content of DSM 32292 initially added to *silage* is not achievable by analysis, the EURL cannot evaluate nor recommend any method for official control for the determination of *Pediococcus pentosaceus* DSM 32292 in *silage*.

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

Pediococcus pentosaceus DSM 32292, technological additives, silage additives, all animal species.

1. BACKGROUND

In the current application authorisation is sought under Article 4(1) for *Pediococcus pentosaceus* DSM 32292 under the category/functional group 1(k) "technological additives"/"silage additives", according to Annex I of Regulation (EC) No 1831/2003 [1]. The authorisation is sought for the use of the *feed additive* for all animal species [1,2].

According to the Applicant, the *feed additive* contains as *active substance* viable cells of the strains *Pediococcus pentosaceus* DSM 32292. The *feed additive* is to be marketed as

preparation with a minimum content of 1×10^{10} Colony Forming Units (CFU) of *Pediococcus pentosaceus* /g *feed additive* [2].

The strain is deposited at the Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH - Germany [2].

The *feed additive* is intended to be used dry or via a water suspension by adding viable freeze-dried bacterial cells of *Pediococcus pentosaceus* DSM 32292 to silage at a minimum dose of 5×10^7 CFU/kg fresh *silage* [2].

Note: The EURL previously evaluated the analytical methods for the determination of *Pediococcus pentosaceus* in the frame of other dossiers [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 *Pediococcus pentosaceus* DSM 32292 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 *Pediococcus pentosaceus* DSM 32292 in the *feed additive*, the Applicant submitted the ring-trial validated spread plate method EN 15786 [4,5].

The sample is suspended and diluted in a buffer solution; the appropriate dilutions are then spread on MRS (de Man, Rogosa, Sharp) agar plates. The agar plates are incubated at 37 °C for 48 h before enumeration. The following performance characteristics of the CEN method, expressed in terms of precision, were calculated after logarithmic transformation of the CFU values [5]:

- a standard deviation for repeatability (S_r) ranging from 0.01 to 0.17 \log_{10} CFU/g;
- a standard deviation for reproducibility (S_R) ranging from 0.10 to 0.26 \log_{10} CFU/g.

In addition, the EURL calculated a limit of quantification (LOQ) of 3×10^3 CFU/g following the recommendations of the ISO 7218 standard [6].

Based on the above-mentioned performance characteristics, the EURL recommends for official control the ring-trial validated EN 15786 method for the enumeration of *Pediococcus pentosaceus* DSM 32292 in the *feed additive*.

The Applicant did not provide any experimental method or data for the determination of *Pediococcus pentosaceus* DSM 32292 in *silage*. Furthermore, the unambiguous determination of the content DSM 32292 initially added to *silage* is not achievable by analysis. Therefore, the EURL cannot evaluate nor recommend any method for official control to determine *Pediococcus pentosaceus* DSM 32292 in *silage*.

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)

The 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) – (Include this section ONLY when relevant for the dossier evaluation e.g. probiotics)

For the taxonomic identification of *Pediococcus pentosaceus* DSM 32292, the Applicant performed a Whole Genome Sequencing Analysis (WGS) [7].

In former reports, for similar dossiers, the EURL recommended for official control Pulsed-Field Gel Electrophoresis (PFGE), a generally recognised methodology for the genetic identification of bacterial strains [3]. The method has been recently ring trial validated and is supposed to become a CEN Technical specification [8].

The EURL considers that both methodologies are fit for purpose for the bacterial identification of authorised additives at a strain level.

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) N 378/2005, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of these authorisations the EURL recommends for official control (i) Whole Genome Sequencing (WGS) or Pulsed-Field Gel Electrophoresis (PFGE) for the identification of *Pediococcus pentosaceus* DSM 32292; and (ii) the ring-trial validated spread plate CEN method (EN 15786) for the enumeration of *Pediococcus* DSM 32292 in the *feed additive*.

Recommended text for the register entry (analytical method)

- Identification: Whole Genome Sequencing (WGS) or Pulsed-Field Gel Electrophoresis (PFGE)
- Enumeration in the *feed additive*: Spread plate method on MRS agar (EN 15786)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Pediococcus pentosaceus* DSM 32292 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. 1831-0059-2021
- [2] *Technical dossier, Section II: II.5 Conditions of use of the additive
- [3] EURL Evaluation Reports related to *Pediococcus* spp:
<https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2009-0024.pdf>
<https://ec.europa.eu/jrc/sites/default/files/finrep-fad-2017-0025-pediococcus-pent.pdf>
- [4] *Technical dossier, Section II: 2.6 Methods of analysis
- [5] EN 15786:2009 “Animal feeding stuffs - Isolation and enumeration of *Pediococcus* spp.”
- [6] EN ISO 7218:2007 - Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations
- [7] *Technical dossier, Annex II_2.1
- [8] CEN project TC 327 WI00327127 (2020): DNA fingerprinting of lactobacilli, pediococci, enterococci and bacilli in animal feeds by pulsed field gel electrophoresis (PFGE) Draft Report of a validation trial

*Refers to Dossier n°: FAD-2021-0074

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation is the “Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA)”, Torino, Italy. 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)