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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

Pediococcus pentosaceus DSM 32291

(FAD-2017-0025; CRL/170010)

European Union Reference Laboratory
Feed Additives

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Dossier related to:	**FAD-2017-0025 - CRL/170010**
Name of Product:	***Pediococcus pentosaceus* DSM 32291**
Active Agent (s):	**Pediococcus pentosaceus DSM 32291**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **JRC Geel, Belgium**
Report prepared by:	**María José González de la Huebra**
Report checked by: Date:	**Piotr Robouch (EURL-FA)** **14/09/2017**
Report approved by: Date:	**Christoph von Holst** **15/09/2017**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4(1) for *Pediococcus pentosaceus* DSM 32291 under the category/functional group 1(k) "technological additives"/"silage additives", according to Annex I of Regulation (EC) No 1831/2003. Specifically, 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 pentosaceus* DSM 32291. The *feed additive* is to be marketed as a preparation containing a minimum *Pediococcus pentosaceus* DSM 32291 content of $8x10^{10}$ Colony Forming Units (CFU)/g. The *feed additive* is intended to be added dry or wet via a water suspension to silage at a minimum dose of $5x10^{7}$ CFU/kg fresh *silage*.

For the identification of *Pediococcus pentosaceus* DSM 32291, the EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised methodology for microbial identification.

For the enumeration of *Pediococcus pentosaceus* DSM 32291 in *feed additive*, the Applicant submitted the ring-trial validated spread plate method EN 15786:2009. Based on the performance characteristics available, the EURL recommends this method for official control.

The Applicant did not provide any experimental method or data for the quantification of *Pediococcus pentosaceus* DSM 32291 in *silage*. Since the unambiguous determination of the content of *Pediococcus pentosaceus* DSM 32291 initially added to *silage* is not achievable by analysis, the EURL cannot evaluate nor recommend any method for official control to quantify the *active substance* 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 32291, technological additives, silage additives, all species

1. BACKGROUND

In the current application authorisation is sought under Article 4(1) for *Pediococcus pentosaceus* DSM 32291 under the category/functional group 1(k) "technological additives"/"silage additives", according to Annex I of Regulation (EC) No 1831/2003 [1].

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 strain *Pediococcus pentosaceus* DSM 32291. The strain is deposited at DSMZ German Collection of Microorganisms and Cell Cultures under accession number *Pediococcus pentosaceus* DSM 32291 [2][3].

The *feed additive* is to be marketed as a preparation containing a minimum *Pediococcus pentosaceus* DSM 32291 content of $8x10^{10}$ Colony Forming Units (CFU)/g [4].

The *feed additive* is intended to be added dry or via a water suspension to *silage* at a minimum dose of $5x10^{7}$ CFU/kg fresh *silage* [2][5].

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 3229 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 *Pediococcus pentosaceus* DSM 32291, the Applicant used a combination of methods: 16S ribosomal testing and Multi Locus Sequence Typing (MLST) [3]. The EURL recommends instead for official control the Pulsed Field Gel Electrophoresis (PFGE), a generally recognised methodology for microbial identification [6]. A PFGE method for microbial identification of authorised probiotics at strain level in *feedingstuffs* [6] is currently being evaluated by the CEN Technical Committee 327 to become an European Standard.

Qualitative and quantitative composition of impurities in the additive

The Applicant analysed the *feed additive* for microbial contaminants (e.g. yeast and mould presumptive *Escherichia coli*, presumptive *Coliforms* and *Salmonella*) using the methods mentioned in the technical dossier [7]. As for the determination of other undesirable substances in the *feed additive* (e.g. arsenic, cadmium, lead, mercury, mycotoxins), analytical methods for official control are available from the respective European Union Reference Laboratories [8][9].

Description of the analytical methods for the determination of the active substance in feed additive and silage

For the enumeration of *Pediococcus pentosaceus* DSM 32291 in the *feed additive*, the Applicant submitted the ring-trial validated spread plate method EN 15786:2009 [10][11].

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 hours before enumeration. The following performance characteristics of the CEN method, expressed in terms of precision, were calculated after logarithmic transformation of the CFU values [11]:

- 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 $3x10^3$ CFU/g following the recommendations of the ISO 7218 standard [12].

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 32291 in the *feed additive per se*.

The Applicant did not provide any experimental method or data for the determination of *Pediococcus pentosaceus* DSM 32291 in silage. Furthermore, the unambiguous determination of the content of *Pediococcus pentosaceus* DSM 32291 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 32291 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.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control the CEN method (EN 15786) for the enumeration of the active agent *Pediococcus pentosaceus* DSM 32291 in the *feed additive* and Pulsed Field Gel Electrophoresis (PFGE) for its identification.

The Applicant did not provide any experimental method or data for the determination of *Pediococcus pentosaceus* DSM 32291 in *silage*. Furthermore, the unambiguous determination of the content of *Pediococcus pentosaceus* DSM 32291 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 32291 in *silage*.

Recommended text for the register entry (analytical method)

- Identification: 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 32291 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. 1831/0016-2017

[2] *Application, Proposal for Register Entry – Annex A

[3] *Technical dossier, Section II: 2.2 Characterisation of the Active Ingredient

[4] *Application Form, Annex 1 of Regulation 429/2008

[5] *Technical dossier, Section II: II.5 Conditions of use of the additive

[6] European Community Project SMT4-CT98-2235."Methods for the Official Control of Probiotics Used as Feed Additives", Report 20873/1 EN (2002) ISBN 92-894-6250-7 (Vol. I) and Report 20873/3 EN (2002) ISBN 92-894-6252-3 (Vol. III)

[7] *Technical dossier, Section II: 2.1.4 Purity

[8] 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

[9] *Technical dossier, Annex II.2

[10] *Technical dossier, Section II: 2.6 Methods of analysis

[11] EN 15786:2009 "Animal feeding stuffs - Isolation and enumeration of *Pediococcus spp.*"

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

*Refers to Dossier no: FAD-2017-0025

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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- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)