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JOINT RESEARCH CENTRE

Directorate F - Health, Consumers and Reference Materials (Geel)  
Food and Feed Compliance



JRC F.5/CvH/MGH/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 IMI 507024**  
*(FAD-2020-0076; CRL/200053)*

**Pediococcus pentosaceus IMI 507025**  
*(FAD-2020-0077; CRL/200054)*





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

Dossier related to: **FAD-2020-0076 - CRL/200053**  
**FAD-2020-0077 – CRL/200054**

Name of Product: ***Pediococcus pentosaceus* IMI 507024**  
***Pediococcus pentosaceus* IMI 507025**

Active Agent (s): ***Pediococcus pentosaceus* IMI 507024**  
***Pediococcus pentosaceus* IMI 507025**

Rapporteur Laboratory: **European Union Reference Laboratory for  
Feed Additives (EURL-FA)**  
**JRC Geel, Belgium**

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Date: **15/03/2021**

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Date: **16/03/2021**

## EXECUTIVE SUMMARY

In the current applications authorisations are sought under Article 4(1) for *Pediococcus pentosaceus* IMI 507024 (FAD 2020-0076) and for *Pediococcus pentosaceus* IMI 507025 (FAD 2020-0077) under the category/functional group 1(k) "technological additives"/"silage additives", according to Annex I of Regulation (EC) No 1831/2003. Specifically, the authorisations are sought for the use of the *feed additives* in *silage* for all animal species.

According to the Applicant, the *feed additives* contain as *active substance* viable cells of the strains *Pediococcus pentosaceus* IMI 507024 or *Pediococcus pentosaceus* IMI 507025. The *feed additives* are to be marketed as preparations with a minimum content of  $1 \times 10^{10}$  Colony Forming Units (CFU) of *Pediococcus pentosaceus* IMI 507024 or *Pediococcus pentosaceus* IMI 507025 / g *feed additive*. Both *feed additives* are intended to be used at a minimum dose of  $1 \times 10^6$  CFU/kg fresh *silage*.

For the identification of *Pediococcus pentosaceus* IMI 507024 and *Pediococcus pentosaceus* IMI 507025, the EURL recommends for official control Pulsed-Field Gel Electrophoresis (PFGE), a generally recognised methodology for the genetic identification of bacterial strains.

For the enumeration of *Pediococcus pentosaceus* IMI 507024 and *Pediococcus pentosaceus* IMI 507025 in the *feed additives*, the EURL recommends for official control the ring-trial validated spread plate method EN 15786.

Since the unambiguous determination of the content of *Pediococcus pentosaceus* IMI 507024 or *Pediococcus pentosaceus* IMI 507025 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* IMI 507024 or *Pediococcus pentosaceus* IMI 507025 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* IMI 507024, *Pediococcus pentosaceus* IMI 507025, technological additives, silage additives, all animal species.

## 1. BACKGROUND

In the current applications authorisations are sought under Article 4(1) (new feed additive) for *Pediococcus pentosaceus* IMI 507024 and *Pediococcus pentosaceus* IMI 507025 under the

category/functional group 1(k) "technological additives"/"silage additives", according to Annex I of Regulation (EC) No 1831/2003 [1,2]. Specifically, the authorisations are sought for the use of the *feed additives* in *silage* for all animal species [1,2].

According to the Applicant, the *feed additives* contain as *active substance* viable cells of the strains *Pediococcus pentosaceus* IMI 507024 or *Pediococcus pentosaceus* IMI 507025. The *feed additives* are to be marketed as preparations with a minimum content of  $1 \times 10^{10}$  Colony Forming Units (CFU) of *Pediococcus pentosaceus* IMI 507024 or *Pediococcus pentosaceus* IMI 507025 /g *feed additive* [3].

Both *feed additives* are intended to be used by adding viable freeze-dried bacterial cells of *Pediococcus pentosaceus* IMI 507024 or *Pediococcus pentosaceus* IMI 507025 directly to silage (granular application) or by dissolving the dried bacterial cells in water and spraying onto silage (liquid application) at a minimum dose of  $1 \times 10^6$  CFU/kg fresh *silage* [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* IMI 507024 and *Pediococcus pentosaceus* IMI 507025 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* IMI 507024 and *Pediococcus pentosaceus* IMI 507025, respectively in the *feed additives*, 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  $3 \times 10^3$  CFU/g following the recommendations of the standard ISO 7218 [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* IMI 507024 and *Pediococcus pentosaceus* IMI 507025 in the *feed additives*.

The Applicant did not provide any experimental method or data for the determination of *Pediococcus pentosaceus* IMI 507024 or *Pediococcus pentosaceus* IMI 507025 in *silage*. Furthermore, the unambiguous determination of the content of *Pediococcus pentosaceus* IMI 507024 or *Pediococcus pentosaceus* IMI 507025 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* IMI 507024 or *Pediococcus pentosaceus* IMI 507025 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)***

For the taxonomic identification of *Pediococcus pentosaceus* IMI 507024 or *Pediococcus pentosaceus* IMI 507025, the Applicant used the sequence analysis of the 16S ribosomal RNA gene [7].

The EURL recommends instead for official control Pulsed-Field Gel Electrophoresis (PFGE), a generally recognised methodology for the genetic identification of bacterial strains [8]. This methodology for bacterial identification of authorised additives at a strain level is currently being evaluated by the CEN Technical Committee 327 to become a European Standard.

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 these authorisations the EURL recommends for official control (i) Pulsed-Field Gel Electrophoresis (PFGE) for the identification of *Pediococcus pentosaceus* IMI 507024 or *Pediococcus pentosaceus* IMI 507025; and (ii) the ring-trial validated spread plate CEN method (EN 15786) for the enumeration of *Pediococcus pentosaceus* IMI 507024 or *Pediococcus pentosaceus* IMI 507025 in the *feed additives*.

##### ***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* IMI 507024 and *Pediococcus pentosaceus* IMI 507025 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. 1831-0067-2020 & Annex I – submission number 1601453190326-2678
- [2] +Application, Reference SANTE/E5: FORW.APPL. 1831-0066-2020 & Annex I – submission number 1602143099546-2690
- [3] \*+Technical dossier, Section II: II.5 Conditions of use of the additive
- [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] 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)

\*Refers to Dossier no: FAD-2020-0076

+Refers to Dossier no: FAD-2020-0077

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## **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**

The following National Reference Laboratories contributed to this report:

- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Centre wallon de Recherches agronomiques (CRA-W), Gembloux (BE)
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, Pesca, Alimentació i Medi Natural. Generalitat de Catalunya, Cabrils (ES)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)