



EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

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



JRC F.5/CvH/ZE/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**

Lactobacillus rhamnosus IMI 507023
Lactobacillus plantarum IMI 507026
Lactobacillus plantarum IMI 507027
Lactobacillus plantarum IMI 507028

(FAD-2020-0075; CRL/200052)

(FAD-2020-0078; CRL/200055)

(FAD-2020-0079; CRL/200056)

(FAD-2020-0080; CRL/200057)



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Dossiers related to: **FAD-2020-0075 - CRL/200052**
FAD-2020-0078 – CRL/200055
FAD-2020-0079 – CRL/200056
FAD-2020-0080 – CRL/200057

Name of Product: ***Lactobacillus rhamnosus IMI 507023***
Lactobacillus plantarum IMI 507026
Lactobacillus plantarum IMI 507027
Lactobacillus plantarum IMI 507028

Active Agent (s): **Lactobacillus rhamnosus IMI 507023**
Lactobacillus plantarum IMI 507026
Lactobacillus plantarum IMI 507027
Lactobacillus plantarum IMI 507028

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

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Date: **04/03/2021**

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Date: **05/03/2021**

EXECUTIVE SUMMARY

In the current applications authorisations are sought under Article 4(1) for *Lactobacillus rhamnosus* IMI 507023, *Lactobacillus plantarum* IMI 507026, *Lactobacillus plantarum* IMI 507027 and *Lactobacillus plantarum* IMI 507028 under the category/functional group 1(k) "technological additives"/"silage additives", according to Annex I of Regulation (EC) No 1831/2003. The authorisations are sought for the use of the *feed additives* for all animal species.

According to the Applicant, the *feed additives* contain as *active substance* viable cells of *Lactobacillus rhamnosus* IMI 507023, *Lactobacillus plantarum* IMI 507026, *Lactobacillus plantarum* IMI 507027 and *Lactobacillus plantarum* IMI 507028, respectively. The *feed additives* are to be marketed as preparations containing a minimum content of 1×10^{10} Colony Forming Units (CFU) of *Lactobacillus rhamnosus* IMI 507023 or *Lactobacillus plantarum* IMI 507026 or *Lactobacillus plantarum* IMI 507027 or *Lactobacillus plantarum* IMI 507028/g in the respective *feed additive*. The *feed additives* are intended to be used at a minimum dose of 1×10^6 CFU/kg fresh *silage*.

For the identification of *Lactobacillus rhamnosus* IMI 507023, *Lactobacillus plantarum* IMI 507026, *Lactobacillus plantarum* IMI 507027 and *Lactobacillus plantarum* IMI 507028 the EURL recommends for official control Pulsed-Field Gel Electrophoresis (PFGE), a recognised methodology for the genetic identification of bacterial strains.

For the enumeration of *Lactobacillus rhamnosus* IMI 507023, *Lactobacillus plantarum* IMI 507026, *Lactobacillus plantarum* IMI 507027 and *Lactobacillus plantarum* IMI 507028 in the *feed additives* the EURL recommends for official control the ring-trial validated spread plate method EN 15787.

Since the unambiguous determination of the content of *Lactobacillus rhamnosus* IMI 507023 or *Lactobacillus plantarum* IMI 507026 or *Lactobacillus plantarum* IMI 507027 or *Lactobacillus plantarum* IMI 507028 initially added to *silage* is not experimentally achievable, the EURL is not able to evaluate or recommend any method for official control for the determination *Lactobacillus rhamnosus* IMI 507023, *Lactobacillus plantarum* IMI 507026, *Lactobacillus plantarum* IMI 507027 and *Lactobacillus plantarum* IMI 507028 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

Lactobacillus rhamnosus IMI 507023, *Lactobacillus plantarum* IMI 507026, *Lactobacillus plantarum* IMI 507027, *Lactobacillus plantarum* IMI 507028, technological additives, silage additives, all animal species.

1. BACKGROUND

In the current applications authorisations are sought under Article 4(1) (new feed additive) for *Lactobacillus rhamnosus* IMI 507023, *Lactobacillus plantarum* IMI 507026, *Lactobacillus plantarum* IMI 507027 and *Lactobacillus plantarum* IMI 507028 under the category/functional group 1(k) "technological additives"/"silage additives", according to Annex I of Regulation (EC) No 1831/2003 [1-4]. The authorisations are sought for the use of the *feed additives* for all animal species [1-4].

According to the Applicant, the *feed additives* contain as *active substance* viable cells of *Lactobacillus rhamnosus* IMI 507023, *Lactobacillus plantarum* IMI 507026, *Lactobacillus plantarum* IMI 507027 and *Lactobacillus plantarum* IMI 507028, respectively [5,6]. The *feed additives* are to be marketed as preparations containing a minimum of 1×10^{10} Colony Forming Units (CFU) of *Lactobacillus rhamnosus* IMI 507023 or *Lactobacillus plantarum* IMI 507026 or *Lactobacillus plantarum* IMI 507027 or *Lactobacillus plantarum* IMI 507028/g *feed additive* [5,6]. The *feed additives* are intended to be used as viable freeze-dried bacterial cells of *Lactobacillus rhamnosus* IMI 507023 or *Lactobacillus plantarum* IMI 507026 or *Lactobacillus plantarum* IMI 507027 or *Lactobacillus plantarum* IMI 507028 directly added to *silage* (granular application) or by dissolving the dried cells in water and spraying onto *silage* (liquid application) at a minimum dose of 1×10^6 CFU/kg fresh *silage* [6].

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 *Lactobacillus rhamnosus* IMI 507023, *Lactobacillus plantarum* IMI 507026, *Lactobacillus plantarum* IMI 507027 and *Lactobacillus plantarum* IMI 507028 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 *Lactobacillus rhamnosus* IMI 507023, *Lactobacillus plantarum* IMI 507026, *Lactobacillus plantarum* IMI 507027 and *Lactobacillus plantarum* IMI 507028, respectively, in the *feed additives*, the Applicant submitted the ring-trial validated spread plate method EN 15787 [7].

According to the protocol of the method, the sample is suspended in phosphate buffered saline and further diluted in peptone salt solution; the appropriate dilutions are then spread on MRS (de Man, Rogosa, Sharp) agar plates. The agar plates are incubated anaerobically at 37 °C for 48 to 72 h [7].

The performance characteristics reported from the validation study of the EN 15787 method after logarithmic transformation of the CFU values [10] are the following: a standard deviation for *repeatability* (S_r) of 0.24 log₁₀ CFU/g and a standard deviation for *reproducibility* (S_R) ranging from 0.29 to 0.38 log₁₀ CFU/g [7].

In addition, following the recommendations of ISO 7218 [8], the EURL calculated a limit of quantification (LOQ) of 3x10³ CFU/g.

Based on the above mentioned performance characteristics, the EURL recommends for official control the ring-trial validated EN 15787 method for the enumeration of *Lactobacillus rhamnosus* IMI 507023, *Lactobacillus plantarum* IMI 507026, *Lactobacillus plantarum* IMI 507027 and *Lactobacillus plantarum* IMI 507028 in the *feed additives*.

The Applicant did not provide any experimental method or data for the determination of *Lactobacillus rhamnosus* IMI 507023, *Lactobacillus plantarum* IMI 507026, *Lactobacillus plantarum* IMI 507027 and *Lactobacillus plantarum* IMI 507028 in *silage*. Furthermore, the unambiguous determination of the content of *Lactobacillus rhamnosus* IMI 507023 or *Lactobacillus plantarum* IMI 507026 or *Lactobacillus plantarum* IMI 507027 or *Lactobacillus plantarum* IMI 507028 initially added to *silage* is not experimentally achievable. Therefore, the EURL is not able to evaluate or recommend any method for official control for the determination *Lactobacillus rhamnosus* IMI 507023, *Lactobacillus plantarum* IMI 507026, *Lactobacillus plantarum* IMI 507027 and *Lactobacillus plantarum* IMI 507028 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)

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 taxonomic identification of *Lactobacillus rhamnosus* IMI 507023, *Lactobacillus plantarum* IMI 507026, *Lactobacillus plantarum* IMI 507027 and *Lactobacillus plantarum* IMI 507028, respectively, the Applicant used the sequence analysis of the 16S ribosomal RNA gene [9].

The EURL recommends instead for official control Pulsed-Field Gel Electrophoresis (PFGE), a recognised methodology for the genetic identification of bacterial strains [10]. 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 the official control: (i) Pulsed-Field Gel Electrophoresis (PFGE) for the identification of *Lactobacillus rhamnosus* IMI 507023, *Lactobacillus plantarum* IMI 507026, *Lactobacillus plantarum* IMI 507027 and *Lactobacillus plantarum* IMI 507028, respectively; and (ii) the ring-trial validated spread plate method EN 15787 for the enumeration of *Lactobacillus rhamnosus* IMI 507023, *Lactobacillus plantarum* IMI 507026, *Lactobacillus plantarum* IMI 507027 and *Lactobacillus plantarum* IMI 507028 in the *feed additives*.

Recommended text for the register entry (analytical method)

- Identification: Pulsed-Field Gel Electrophoresis (PFGE)
- Enumeration in the *feed additives*: Spread plate method on MRS agar (EN 15787)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Lactobacillus rhamnosus* IMI 507023, *Lactobacillus plantarum* IMI 507026, *Lactobacillus plantarum* IMI 507027 and *Lactobacillus plantarum* IMI 507028 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] ^a Application, Reference SANTE/E5: FORW.APPL. 1831-0068-2020 & Annex I – submission number 1601452456390-2676
- [2] ^b Application, Reference SANTE/E5: FORW.APPL. 1831-0065-2020 & Annex I – submission number 1601907778839-2688
- [3] ^c Application, Reference SANTE/E5: FORW.APPL. 1831-0064-2020 & Annex I – submission number 1601453583354-2680
- [4] ^d Application, Reference SANTE/E5: FORW.APPL. 1831-0063-2020 & Annex I – submission number 1601839970394-2686
- [5] ^{a,b,c,d} Technical dossier, Section II: 2.1.3. Qualitative and quantitative composition (active substance/agent, other components, impurities, batch to batch variation)
- [6] ^{a,b,c,d} Technical dossier, Section II: 2.5.1. Proposed mode of use in animal nutrition
- [7] EN 15787 - Animal feeding stuffs - Isolation and enumeration of *Lactobacillus spp.*
- [8] EN ISO 7218:2007 - Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations
- [9] ^{a,b,c,d} Technical dossier, Section II – Annex II_2_3
- [10] 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)

^a Refers to Dossier no: FAD-2020-0075

^b Refers to Dossier no: FAD-2020-0078

^c Refers to Dossier no: FAD-2020-0079

^d Refers to Dossier no: FAD-2020-0080

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:

- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Centre wallon de Recherches agronomiques (CRA-W), Gembloux (BE)

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- Instytut Zootechniki — Państwowy Instytut Badawczy, Krajowe Laboratorium Pasz, Lublin (PL)
 - Univerza v Ljubljani. Veterinarska fakulteta. Nacionalni veterinarski inštitut. Enota za patologijo prehrane in higieno okolja, Ljubljana (SI)
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