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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**

Lactococcus lactis DSM 34262
(FEED-2023-13050; CRL/230007)



**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**

Dossier related to: **FEED-2023-13050 - CRL/230007**

Name of Product : ***Lactococcus lactis DSM 34262***

Active Agent (s): **Lactococcus lactis DSM 34262**

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

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Date: **28/08/2023**

Report approved by: **Christoph von Holst**
Date: **29/08/2023**

EXECUTIVE SUMMARY

In the current application an authorisation is sought under Article 4(1) for *Lactococcus lactis* DSM 34262 under the category / functional group 1(k) 'technological additives' / 'silage additives', according to Annex I of Regulation (EC) No 1831/2003. The authorisation is sought for the use of the *feed additive* for all animal species.

According to the Applicant, the *feed additive* contains the non-genetically modified strain *Lactococcus lactis* DSM 34262 as *active substance* at a minimum level of 3×10^{11} Colony Forming Unit (CFU) / g. The *feed additive* is intended to be added to the forage, used for ensiling process at a minimum recommended dosage of 1×10^5 CFU /g fresh *silage*.

For the enumeration of *Lactococcus lactis* DSM 34262 in the *feed additive* and *silage* the Applicant applied an in-house pour-plate method based on the ISO 15214 pour-plate method.

Based on the available information, the EURL recommends for official control the ISO 15214 method for the enumeration of *Lactococcus lactis* DSM 34262 in the *feed additive per se*.

Furthermore, for the identification of *Lactococcus lactis* DSM 34262 at a strain level, the EURL recommends for official control (i) DNA sequencing methods (e.g. Whole Genome Sequencing (WGS)) or (ii) Pulsed-Field Gel Electrophoresis (PFGE).

As the enumeration of added *Lactococcus lactis* DSM 34262 in *silage* is not achievable by analysis, the EURL cannot recommend any method for official control.

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

Lactococcus lactis DSM 34262, technological additives, silage additives, all species.

1. BACKGROUND

In the current application an authorisation is sought under Article 4(1) (new feed additive) for *Lactococcus lactis* DSM 34262 under the category / functional group 1(k) 'technological additives' / 'silage 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 all animal species [1,2].

According to the Applicant, the *feed additive* contains *Lactococcus lactis* DSM 34262 as *active substance* at a minimum concentration of 3×10^{11} Colony Forming Unit (CFU) / g [3].

The Applicant stated that the *Lactococcus lactis* DSM 34262 is a non-genetically modified strain. The microorganism is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) [4].

The *feed additive* is intended to be added to the forage, used for ensiling process at a minimum recommended dosage of 1×10^5 CFU / g fresh *silage* [5].

Note: The EURL has previously evaluated the analytical methods for the determination of other *Lactococcus lactis* strains in the frame of several dossiers [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 *Lactococcus lactis* DSM 34262 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, compound feed and when appropriate water (section 2.6.1 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

For the enumeration of *Lactococcus lactis* DSM 34262 in the *feed additive* and *silage* the Applicant applied an in-house pour-plate method [7] based on the ISO 15214 pour-plate method [8].

The ISO 15214 method is dedicated for the enumeration of mesophilic lactic acid bacteria in food and feed. Following the method, a sample is suspended and diluted in a buffer solution. After appropriate dilutions the solutions are poured on plates filled with MRS (de Man, Rogosa, Sharp) agar at pH 5.7. The plates are incubated at 30 °C for 72 h [8].

The Applicant demonstrated the fitness-for-purpose of the proposed method by providing experimental data obtained for the *feed additive* in the frame of the batch-to-batch variation [9] and in the stability studies [10].

Based on the available information, the EURL recommends for official control the ISO 15214 method for the enumeration of *Lactococcus lactis* DSM 34262 in the *feed additive per se*.

As the unambiguous determination of *Lactococcus lactis* DSM 34262 added to silage is not achievable by analysis, the EURL cannot evaluate nor recommend the ISO 15214 or any other method for official control to enumerate *Lactococcus lactis* DSM 34262 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 identification of *Lactococcus lactis* DSM 34262, the Applicant applied a DNA sequencing method such as Whole Genome Sequencing (WGS) [4].

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 [6]. The method has been ring-trial validated and recently published as a CEN Technical specification [11].

The EURL considers that both methodologies are suitable for official control for the bacterial identification of *Lactococcus lactis* DSM 34262 at 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) 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 (i) DNA sequencing methods (e.g. Whole Genome Sequencing (WGS)) or Pulsed-Field Gel Electrophoresis (PFGE) for the identification of *Lactococcus lactis* DSM 34262 and (ii) the pour-plate ISO method (ISO 15214) for the enumeration of *Lactococcus lactis* DSM 34262 in the feed additive.

As the unambiguous determination of *Lactococcus lactis* DSM 34262 added to silage is not achievable by analysis, the EURL cannot recommend the ISO 15214 or any other method for official control to enumerate *Lactococcus lactis* DSM 34262 in silage.

Recommended text for the register entry (analytical method)

- Identification: DNA sequencing methods or Pulsed-Field Gel Electrophoresis (PFGE)
- Enumeration in the feed additive: Pour-plate method on MRS agar (ISO 15214)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Lactococcus lactis* DSM 34262 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] Forwarding of applications for authorisation of feed additives in accordance with Regulation (EC) No 1831/2003 – E-Submission Food Chain platform
<https://webgate.ec.europa.eu/esfc/#/applications/38510>
<https://open.efsa.europa.eu/questions/EFSA-Q-2023-00249>
 - [2] *Application, Annex 1
 - [3] *Technical dossier, Section II: 2.1 Identity of the additive
 - [4] *Technical dossier, Section 2.2 Characterisation of the Microorganisms
 - [5] *Technical dossier, Section II: 2.5 Conditions of use of the additive
 - [6] EURL reports:
<https://joint-research-centre.ec.europa.eu/system/files/2013-02/FinRep-FAD-2010-0032.pdf>
https://joint-research-centre.ec.europa.eu/system/files/2015-05/finrep-fad-2013-0048_lavipan_online.pdf
<https://joint-research-centre.ec.europa.eu/system/files/2020-10/finrep-fad-2019-0090-mixbalac.pdf>
<https://joint-research-centre.ec.europa.eu/system/files/2013-02/FinRep-uorg-silage-group1.pdf>
 - [7] *Technical dossier, Annex_II_18_Method_Inhouse_confid.pdf
 - [8] ISO 15214:1998 – Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of mesophilic lactic acid bacteria
 - [9] *Technical dossier, Annex_II_2_Batch-to-batch.pdf
 - [10] *Technical dossier, Annex_II_13 & Annex_II_14
 - [11] CEN/TS 17697:2023 - Animal feeding stuffs - Methods of sampling and analysis - PFGE typing of Lactobacilli, Pediococci, Enterococci and Bacilli in animal feeds
- *Refers to Dossier no:FEED-2023-13050

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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- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Laboratoire de Rennes (SCL L35), Service Commun des Laboratoires DGCCRF et DGDDI, Rennes (FR)