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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

Lactobacillus casei DSM 28872
(FAD-2016-0016; CRL/160006)

European Union Reference Laboratory
Feed Additives

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:	**FAD-2016-0016 - CRL/160006**
Name of Product:	***Lactobacillus casei DSM 28872***
Active Agent:	**Lactobacillus casei DSM 28872**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **JRC, Geel, Belgium**
Report prepared by:	**Stefano Bellorini**
Report checked by: Date:	**Piotr Robouch (EURL-FA)** **27/10/2016**
Report approved by: Date:	**Christoph von Holst** **27/10/2016**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4(1) for *Lactobacillus casei DSM 28872* 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 non-genetically modified strain *Lactobacillus casei DSM 28872*. The *feed additive* is to be marketed as a powder containing a minimum *Lactobacillus casei DSM 28872* content of $8x10^{10}$ Colony Forming Units (CFU)/g. The *feed additive* is intended to be added dry or sprayed onto silage at a minimum dose of $5x10^{7}$ CFU/kg fresh *silage*.

For the identification of *Lactobacillus casei DSM 28872*, the EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised methodology for microbial identification.

For the enumeration of *Lactobacillus casei DSM 28872* in *feed additive*, the Applicant submitted the ring-trial validated spread plate method EN 15787 specifically designed for the analysis of Lactobacillus spp. Based on the performance characteristics available, the EURL recommends for official control this method for the enumeration of *Lactobacillus casei DSM 28872* in the *feed additive per se*.

The Applicant did not provide any experimental method or data for the determination of *Lactobacillus casei DSM 28872* in *silage*. Since the unambiguous determination of the content of *Lactobacillus casei DSM 28872* initially added to *silage* is not achievable by analysis, the EURL cannot evaluate or 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) is not considered necessary.

KEYWORDS

Lactobacillus casei DSM 28872, technological additives, silage additives, all species

1. BACKGROUND

In the current application authorisation is sought under Article 4(1) for *Lactobacillus casei DSM 28872* 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 non-genetically modified strain *Lactobacillus casei DSM 28872*. The strain is deposited at "Deutsche Sammlung von Mikroorganismen und Zellkulturen" (DSMZ) with reference *Lactobacillus casei DSM 28872* [2,3].

The *feed additive* is to be marketed as a powder containing a minimum *Lactobacillus casei DSM 28872* content of $8x10^{10}$ Colony Forming Units (CFU)/g [4,5].

The *feed additive* is intended to be added dry or sprayed onto *silage* at a minimum dose of $5x10^{7}$ CFU/kg fresh *silage* [2,6].

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 casei DSM 28872* 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 *Lactobacillus casei DSM 28872*, 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 [7].

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 [8]. 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 [9].

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

For the enumeration of *Lactobacillus casei DSM 28872* in *feed additive*, the Applicant submitted the ring-trial validated spread plate method EN 15787:2009 specifically designed for the analysis of Lactobacillus spp [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 anaerobically at 37 °C for 48 to 72 hours before enumeration. The reported performance characteristics were calculated after logarithmic transformation of the CFU values obtained in the validation study: (i) a standard deviation for repeatability (S_r) of 0.24 $\log_{10}$ CFU/g [11]; and (ii) a standard deviation for reproducibility (S_R) ranging from 0.29 to 0.38 $\log_{10}$ CFU/g [11]. 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 these performance characteristics, the EURL recommends for official control the ring-trial validated EN 15787 method for the enumeration of *Lactobacillus casei DSM 28872* in *feed additive per se*.

The Applicant did not provide any experimental method or data for the determination of *Lactobacillus casei DSM 28872* in *silage*. Furthermore, the unambiguous determination of the content of *Lactobacillus casei DSM 28872* initially added to *silage* is not achievable by analysis. Therefore, the EURL cannot evaluate nor recommend any method for official control to determine *Lactobacillus casei DSM 28872* 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) is not considered necessary.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control (i) the spread plate method (EN 15787) for the enumeration of the active agent *Lactobacillus casei DSM 28872* in the *feed additive* and (ii) Pulsed Field Gel Electrophoresis (PFGE) for its identification.

The Applicant did not provide any experimental method or data for the determination of *Lactobacillus casei DSM 28872* in *silage*. Furthermore, the unambiguous determination of the content of *Lactobacillus casei DSM 28872* initially added to *silage* is not achievable by analysis. Therefore, the EURL cannot evaluate nor recommend any method for official control to determine *Lactobacillus casei DSM 28872* 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 15787)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Lactobacillus casei DSM 28872* 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 SANCO/G1: Forw. Appl. 1831/0014-2016

[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] *Suppl. Information dated 18/07/2016: Lactobacillus casei DSM 28872.pdf

[6] *Technical dossier, Section II: 2.5 Conditions of use of the additive

[7] 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)

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

[9] 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

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

[11] EN 15787:2009 "Animal feedingstuff - enumeration and isolation of *Lactobacillus 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-2016-0016

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was " 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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