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European Union Reference Laboratory for Feed Additives

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EURL Evaluation Report on the Analytical Methods submitted in connection with the Application for the Authorisation of a new Feed Additive according to Regulation (EC) No 1831/2003

Dossier related to:	FAD-2010-0109 CRL /100106
Product Name:	*Lactobacillus plantarum DSM 21762*
Active Substance(s):	*Lactobacillus plantarum DSM 21762*
Rapporteur Laboratory:	European Union Reference Laboratory for Feed Additives (EURL -FA)
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Date:	17/02/2011
Report approved by:	Christoph von Holst
Date:	28/02//2011

EXECUTIVE SUMMARY

In the current application authorisation is sought for the microbial feed additive *Lactobacillus plantarum DSM 21762* under the category 'technological additives', functional group 'silage additives' according to Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the *feed additive* to be placed on the market in the form of powder containing a minimum concentration of $5x10^{11}$ CFU/g of *Lactobacillus plantarum DSM 21762*. The intended use of the current application is for all animal species. It is proposed be mix the product into silage with water providing a minimum concentration in silage of $1x10^{5}$ CFU/g.

For enumeration of *Lactobacillus plantarum DSM 21762* in *feed additive,* the Applicant submitted a spread plate method based on the ring-trial validated CEN method (EN 15787). The performance characteristics reported after logarithmic transformation of measured values (CFU) are:

- a standard deviation for repeatability (S_r) ranging from 0.24 to 0.24 $\log_{10}$ CFU/g;
- a standard deviation for reproducibility (S_R) ranging from 0.29 to 0.38 $\log_{10}$ CFU/g; and
- a limit of detection (LOD) of 10^{5} CFU/kg of *feedingstuffs.*

Based on the performances characteristics of the method the EURL recommends for official control the EN 15787 for the determination of *Lactobacillus plantarum DSM 21762* in the *feed additive per se*.

The Applicant did not provide any experimental method or data for the determination of *Lactobacillus plantarum DSM 21762* in *silage*. Furthermore, the method available is not able to determine the content of *Lactobacillus plantarum DSM 21762* added to silage. Therefore the EURL cannot evaluate nor recommend any method for official control to determine *Lactobacillus plantarum DSM 21762* in *silage*.

Molecular methods were used by the Applicant for identification of the active agent. The EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised standard methodology for microbial identification.

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 plantarum DSM 21762, technological additives, silage additives.

1. BACKGROUND

Lactobacillus plantarum DSM 21762 is a feed additive for which authorisation under Article 4(1) is sought under the category of 'technological additives' functional group 'k) silage additives' according to Annex I of Regulation (EC) No 1831/2003 [1]. Specifically, authorisation is sought for the *feed additive* to be placed on the market in the form of powder, containing minimum concentration of $5x10^{11}$ CFU/g of *Lactobacillus plantarum DSM 21762* [2]. The original strain is deposited in Deutsche Sammlung von Mikro-organismem und Zelkulturen (DSMZ) [3]. The intended use of the current application is for all animal species. It is proposed to be mix the product into silage via a suspension in water providing a minimum concentration of $1x10^5$ CFU/g in fresh matter ensilage [4].

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005 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 tasks of the European Union Reference Laboratory concerning applications for authorizations of *feed additives*, as last amended by Regulation (EC) No 885/2009, the EURL is requested to submit a full evaluation report to the European Food Safety Authority (EFSA) for each application, or for each group of applications. For this particular dossier, the methods of analysis submitted in connection with the *Lactobacillus plantarum DSM 21762* 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

Qualitative and quantitative composition of the additive

For identification and characterization of the strain *Lactobacillus plantarum DSM 21762* the Applicant used Randomly Amplified Polymorphic DNA-PCR (RAPD-PCR) [5]. This method is suitable for the purpose of analysis. However, the EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised standard methodology for microbial identification [6].

Qualitative and quantitative composition of any impurities in the additive

The Applicant analysed the *feed additive* for microbial contaminants (such as Enterobacteria, *Escherichia coli*, Salmonella spp. and yeasts) by using appropriate EN ISO tests [7]. For

undesirable substances (i.e. arsenic, cadmium, mercury, lead, selenium, copper, zinc, chrome, aflatoxins) internationally recognised standard methods are available at the respective European Union Reference Laboratories, in accordance with Commission Regulation (EC) No 776/2006.

Description of the analytical methods for the determination of active agent(s) in feed additive, premixtures and feedingstuffs

For enumeration of *Lactobacillus plantarum DSM 21762* in *feed additive,* the applicant submitted a spread plate method [8] based on the ring-trial validated CEN method (EN 15787). The sample is suspended and diluted in a buffer solution; the appropriated dilutions are then spread on MRS (de Man, Rogosa, Sharp) agar plates. The agar plates are incubated at 37°C for 48 to 72 hours. The performance characteristics of the EN 15787 method reported after logarithmic transformation of measured values (CFU) are [9]:

- a standard deviation for repeatability (S_r) ranging from 0.24 to 0.24 $\log_{10}$ CFU/g;
- a standard deviation for reproducibility (S_R) ranging from 0.29 to 0.38 $\log_{10}$ CFU/g; and
- a limit of detection (LOD) of 10^5 CFU/kg of *feedingstuffs* [10].

Based on the performances characteristics of the method the EURL recommends for official control the EN 15787 for the determination of *Lactobacillus plantarum DSM 21762* in the *feed additive per se*.

The Applicant did not provide any experimental method or data for the determination of *Lactobacillus plantarum DSM 21762* in *silage*. Furthermore, the method available is not able to determine the content of *Lactobacillus plantarum DSM 21762* **added** to silage. Therefore the EURL cannot evaluate nor recommend any method for official control to determine *Lactobacillus plantarum DSM 21762* 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 EN 15787 method for the enumeration of the active agent *Lactobacillus plantarum DSM 21762* in the *feed additive*.

For the analysis of the identity of the bacterial strain, *Lactobacillus plantarum DSM 21762,* the EURL recommends Pulsed Field Gel Electrophoresis (PFGE) for official control.

The Applicant did not provide any experimental method or data for the determination of *Lactobacillus plantarum DSM 21762* in *silage*. Furthermore, the method available is not able to determine the content of *Lactobacillus plantarum DSM 21762* **added** to silage. Therefore the EURL cannot evaluate nor recommend any method for official control to determine *Lactobacillus plantarum DSM 21762* in *silage*.

Recommended text for the register entry, fourth column (Composition, chemical formula, description, analytical method)

- Enumeration in the feed additive: Pour plate method: EN 15787
- Identification of the feed additive: Pulsed Field Gel Electrophoresis (PFGE)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, samples of the additive *Lactobacillus plantarum DSM 21762,* for all animal species up to slaughter age, have been sent to the European Union Reference Laboratory for Feed Additives Authorisation. The dossier has been made available to the EURL by EFSA.

6. REFERENCES

[1] *Application/Ref: SANCO/D/2: Forw.Appl.1831/0072-2010
[2] *Application, Annex A, Proposal for register entry
[3] *Technical Dossier, Section II.2. Characterisation of the active Substance(s)/ agents(s)
[4] *Technical Dossier, Section II 2.5.1. Conditions of use of the additive
[5] *Technical Dossier, Annex II.2.4 Gen_Stab
[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)"
[7] *Technical Dossier, Section II.1.4. Purity
[8] *Technical Dossier, Section II.6.1. Methods of analysis of the active substance
[9] EN 15787 : " Animal feeding stuffs- Isolation and enumeration of Lactobacillus spp."
[10] ISO 7218:1996, Microbiology of food and animal feedingstuffs – General rules for microbiological examinations

*Refers to Dossier no: FAD-2010-0109

7. RAPPORTEUR LABORATORY

The Rapporteur Laboratory for this evaluation was European Union Reference Laboratory for Feed Additives, IRMM, 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.

8. Acknowledgements

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– Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Austria

– Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Czech Republic

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