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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 buchneri NRRL B-50733
(FAD-2016-0060; CRL/160036)

**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-2016-0060 - CRL/160036**

Name of Product: ***Lactobacillus buchneri* NRRL B-50733**

Active Agent (s): **Lactobacillus buchneri NRRL B-50733**

Rapporteur Laboratory: **Centro di referenza nazionale per la
sorveglianza e il controllo degli alimenti
per gli animali (CReAA), Torino, Italy**

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Date: **(EURL-FA)
06/04/2017**

Report approved by: **Christoph von Holst**
Date: **10/04/2017**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4(1) for *Lactobacillus buchneri* NRRL B-50733 under the category / functional group 1(k) "technological additives" / "silage additives", according to Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for the use of the *feed additive* for all animal species.

According to the Applicant, the *feed additive* contains as active substance viable cells of the non-genetically modified strain *Lactobacillus buchneri* NRRL B-50733. The *feed additive* is to be marketed as a powder, with a minimum *Lactobacillus buchneri* NRRL B-50733 content of 1×10^{10} Colony Forming Units (CFU)/g product. The *feed additive* is intended to be added dry or sprayed onto *silage* at a minimum dose of 1×10^8 CFU/kg fresh *silage*.

For the identification of *Lactobacillus buchneri* NRRL B-50733, the EURL recommends for official control the Pulsed Field Gel Electrophoresis (PFGE), a generally recognised methodology for genetic identification.

For the enumeration of *Lactobacillus buchneri* NRRL B-50733 in the *feed additive*, the Applicant submitted the ring-trial validated spread plate method EN 15787, dedicated specifically 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 buchneri* NRRL B-50733 in the *feed additive per se*.

Since the unambiguous enumeration of *Lactobacillus buchneri* NRRL B-50733 initially added to *silage* is not achievable by analysis, the EURL cannot evaluate nor 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) is not considered necessary.

KEYWORDS

Lactobacillus buchneri NRRL B-50733, technological additives, silage additives, all animal species

1. BACKGROUND

In the current application authorisation is sought under Article 4(1) (new feed additive) for *Lactobacillus buchneri* NRRL B-50733 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 buchneri* NRRL B-50733. The strain is deposited at the International Depository Authority/Agricultural Research Culture Collection (NRRL) with reference to *Lactobacillus buchneri* NRRL B-50733 [2,3].

The *feed additive* is to be marketed as a powder with a minimum *Lactobacillus buchneri* NRRL B-50733 content of 1×10^{10} Colony Forming Units (CFU)/g product [4].

The *feed additive* is intended to be added dry or sprayed onto *silage* at a minimum dose of 1×10^8 CFU/kg fresh *silage* [2,5].

Note: The EURL previously evaluated the analytical methods for the determination of *Lactobacillus spp* 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 *Lactobacillus buchneri* NRRL B-50733 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 buchneri* NRRL B-50733, the Applicant applied sequencing of 16S rDNA gene and Restriction Fragment Length Total DNA Profiling [7]. The EURL recommends instead for official control the Pulsed Field Gel Electrophoresis (PFGE), a generally recognised methodology for genetic identification [8].

Qualitative and quantitative composition of impurities in the additive

The Applicant analysed the *feed additive* for microbial contaminants (e.g. coliforms, yeast and moulds, *Escherichia coli* and *Salmonella*) using the methods described in the technical dossier [9]. As for the determination of the undesirable substances in the *feed additive* (e.g. arsenic, cadmium, lead, mercury, aflatoxin B1 and dioxins), analytical methods are available from the respective European Union Reference Laboratories [10].

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

For the enumeration of *Lactobacillus buchneri* NRRL B-50733 in the *feed additive*, the Applicant submitted the ring-trial validated spread plate method EN 15787:2009, dedicated to the analysis of *Lactobacillus spp* [11].

The sample is suspended and diluted in a buffer solution; the appropriate dilutions are then spread on MRS (de Man, Rogosa and Sharp) agar plates. The agar plates are incubated at 37 °C for 48 to 72 hours before the enumeration of *lactobacilli*. The following performance characteristics were calculated after logarithmic transformation of CFU values obtained in the validation study [11]: (i) a standard deviation for repeatability (S_r) of 0.24 \log_{10} CFU/g; and (ii) a standard deviation for reproducibility (S_R) ranging from 0.29 to 0.38 \log_{10} CFU/g. In addition, the EURL calculated a limit of quantification (LOQ) of 3×10^3 CFU/g of the *feed additive* following the recommendations of the ISO 7218 standard [12].

Based on the performance characteristics available, the EURL recommends for official control the ring-trial validated EN 15787 method for the enumeration of *Lactobacillus buchneri* NRRL B-50733 in the *feed additive per se*.

The applicant did not provide any experimental method or data for the determination of *Lactobacillus buchneri* NRRL B-50733 in *silage*. Furthermore, the unambiguous enumeration of *Lactobacillus buchneri* NRRL B-50733 initially added to *silage* is not achievable by analysis. Therefore, the EURL cannot evaluate nor 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) is not considered necessary.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE) for the identification of *Lactobacillus buchneri* NRRL B-50733 and the spread plate method (EN 15787) for its enumeration in the *feed additive*. Since the unambiguous enumeration of *Lactobacillus buchneri* NRRL B-50733 initially added to silage is not achievable by analysis, the EURL cannot evaluate nor recommend any method for official control to determine *Lactobacillus buchneri* NRRL B-50733 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 buchneri* NRRL B-50733 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. 1831/0045-2016
- [2] *Application, Proposal for Register Entry – Annex A
- [3] *Technical dossier, Section II: 2.2. Characterisation of the active substance(s)/agent(s)
- [4] *Technical dossier, Section II: 2.1.3. Qualitative and quantitative composition (active substance/agent, other components, impurities, batch to batch variation)
- [5] *Technical dossier, Section II: 2.5: Conditions of use of the additive
- [6] EURL Evaluation Reports:
https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2016-0016_lacto_casei.pdf
https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad_2015_0034_lactob_plantarum.pdf
https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2015-0028-lacto_dioliv.pdf
<https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-FAD-2015-0013-lactobacillus%20plantarum.pdf>
<https://ec.europa.eu/jrc/sites/jrcsh/files/FinRep-FAD-2011-0004.pdf>
- [7] *Technical dossier, Section II: 2.2. Characterisation of the active substance(s)/agent(s)
- [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)
- [9] *Technical dossier, Section II: 2.6 Methods of analysis and reference samples
- [10] 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

[11] EN 15787:2009 – Animal feeding stuffs – Isolation and enumeration 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-2016-0060

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation is the Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino, Italy. 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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