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

Dossier related to: **FAD-2012-0018**
CRL/120011

Product name: ***Lactobacillus kefir* BIO 94 IFA 94 - DSM 19455**

Active Substance(s): ***Lactobacillus kefir* BIO 94 IFA 94 - DSM 19455**

Rapporteur Laboratory: **European Union Reference Laboratory
for Feed Additives (EURL-FA)
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Date: **30/10/2012**

Report approved by: **Christoph von Holst**

Date: **31/10/2012**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4(1) for *Lactobacillus kefir* BIO 94 IFA 94 – DSM 19455, under the category/functional group 1(k), "technological additives/silage additives", according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the *feed additive* for all animal species and categories. The *feed additive* is to be placed on the market as a powder, containing a minimum concentration of 1×10^{10} CFU/g of *Lactobacillus kefir* BIO 94 IFA 94 – DSM 19455. It is intended to be mixed directly into silage or suspended in water and sprayed on silage with a minimum concentration of 5×10^7 CFU/kg *fresh forage*.

For enumeration of *Lactobacillus kefir* BIO 94 IFA 94 – DSM 19455 in *feed additive*, the Applicant submitted the ring-trial validated spread plate CEN method (EN 15787), using MRS agar. The performance characteristics of the method reported after logarithmic transformation are:

- a standard deviation for *repeatability* (S_r) of $0.24 \log_{10}$ 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 *feedingstuffs*.

Based on the performances characteristics presented, the EURL recommends for official control, the CEN method (EN 15787) for the determination of *Lactobacillus kefir* BIO 94 IFA 94 – DSM 19455 in the *feed additive per se*.

The Applicant did not provide any experimental method or data for the determination of *Lactobacillus kefir* BIO 94 IFA 94 – DSM 19455 in *silage*. Furthermore, the unambiguous determination of the content of *Lactobacillus kefir* BIO 94 IFA 94 – DSM 19455 added to *silage* is not achievable by analysis. Therefore the EURL cannot evaluate nor recommend any method for official control to determine *Lactobacillus kefir* BIO 94 IFA 94 – DSM 19455 in *silage*.

Molecular methods were used by the Applicant to identify the active agent in the *feed additive*. 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 kefir BIO 94 IFA 94 – DSM 19455, technological additives, silage additives, all animal species and categories.

1. BACKGROUND

In the current application authorisation is sought under Article 4(1) (new authorisation) for *Lactobacillus kefir* BIO 94 IFA 94 – DSM 19455, under the category/functional group 1(k), "technological additives/silage additives", according to the classification system of Annex I of Regulation (EC) No 1831/2003 [1]. Specifically, authorisation is sought for the use of the *feed additive* for all animal species and categories. The *feed additive* is to be placed on the market as a powder, containing a minimum concentration of 1×10^{10} CFU/g of *Lactobacillus kefir* BIO 94 IFA 94 – DSM 19455 [2,3]. *Lactobacillus kefir* BIO 94 IFA 94 – DSM 19455 is deposited in the 'Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ)' in Braunschweig, Germany [4]. It is intended to be mixed directly into silage or suspended in water and sprayed on silage with a minimum concentration of 5×10^7 CFU/kg *fresh forage* [3, 5].

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 authorisations 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 kefir* BIO 94 IFA 94 – DSM 19455 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 characterisation of the strain *Lactobacillus kefir* BIO 94 IFA 94 – DSM 19455 the Applicant used morphological, physiological, biochemical and molecular methods, such as 16S rDNA sequence analysis [6] and Pulsed Field Gel Electrophoresis

(PFGE) [7]. These methods are suitable for the purpose of analysis. The EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised standard methodology for microbial identification [8].

Qualitative and quantitative composition of impurities in the additive

The Applicant analysed the *feed additive* for microbial contaminants (such as coliforms, *Escherichia coli*, *Salmonella* spp., yeasts and moulds) by using appropriate EN ISO and AOAC tests [9]. 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 Laboratory, in accordance with Commission Regulation (EC) No 776/2006.

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

For enumeration of *Lactobacillus kefir* BIO 94 IFA 94 – DSM 19455 in *feed additive*, the Applicant proposed the ring-trial validated spread plate method (EN 15787) [10], developed by CEN for the enumeration of *Lactobacillus* spp. The sample is suspended and diluted in a phosphate buffered saline (PBS); 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. The performance characteristics of the CEN method reported after logarithmic transformation are [8]:

- a standard deviation for *repeatability* (S_r) of 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* [11].

Based on the performances characteristics presented, the EURL recommends for official control the CEN method (EN 15787) for the determination of *Lactobacillus kefir* BIO 94 IFA 94 – DSM 19455 in the *feed additive per se*.

The Applicant did not provide any experimental method or data for the determination of *Lactobacillus kefir* BIO 94 IFA 94 – DSM 19455 in *silage*. Furthermore, the unambiguous determination of the content of *Lactobacillus kefir* BIO 94 IFA 94 – DSM 19455 added to *silage* is not achievable by analysis. Therefore the EURL cannot evaluate nor recommend any method for official control to determine *Lactobacillus kefir* BIO 94 IFA 94 – DSM 19455 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 the CEN method (EN 15787) for the enumeration of *Lactobacillus kefir* BIO 94 IFA 94 – DSM 19455 in the *feed additive* and Pulsed Field Gel Electrophoresis (PFGE) for its identification.

The Applicant did not provide any experimental method or data for the determination of *Lactobacillus kefir* BIO 94 IFA 94 – DSM 19455 in *silage*. Furthermore, the unambiguous determination of the content of *Lactobacillus kefir* BIO 94 IFA 94 – DSM 19455 added to *silage* is not achievable by analysis. Therefore the EURL cannot evaluate nor recommend any method for official control to determine *Lactobacillus kefir* BIO 94 IFA 94 – DSM 19455 in *silage*.

Recommended text for the register entry (analytical method)

- Enumeration of *Lactobacillus kefir* BIO 94 IFA 94 – DSM 19455 in the *feed additive*:
Spread plate method (EN 15787)
- Identification of *Lactobacillus kefir* BIO 94 IFA 94 – DSM 19455:
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 kefir* BIO 94 IFA 94 – DSM 19455 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/Ref: SANCO/G1: Forw.Appl.1831/0037-2012
 - [2] *Application, Annex A, Proposal for register entry
 - [3] *Technical Dossier, Section II, Annex II_01 Annex entry proposal
 - [4] *Technical Dossier, Section II, Annex II_26 DSMZ LK
 - [5] *Technical Dossier, Section II.2.5 Conditions of use of the additive
 - [6] *Technical Dossier, Section II, Annex II_27 Characterisation LK
 - [7] *Technical Dossier, Section II, Annex II_28 Differentiation of BIO 94
 - [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)"
 - [9] *Technical Dossier, Section II.2.1.4. Purity
 - [10] EN 15787:2009 - Animal feeding stuffs- Isolation and enumeration of *Lactobacillus* spp.
 - [11] ISO 7218:1996 - Microbiology of food and animal feedingstuffs – General rules for microbiological examinations
- *Refers to Dossier No: FAD-2012-0018

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

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, as last amended by Regulation (EC) No 885/2009.

8. ACKNOWLEDGEMENTS

The following National Reference Laboratories contributed to this report:

- Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim (DE)
- Instytut Zootechniki w Krakowie, Krajowe Laboratorium Pasz, Lublin (PL)
- Państwowy Instytut Weterynaryjny, Puławy (PL)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen. Jena (DE)
- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes (FR)