EUROPEAN COMMISSION
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European Union Reference Laboratory for Feed Additives

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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 brevis TAK 124-1 NCIMB 42149

(FAD-2015-0014; CRL/150003)

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-2015-0014 - CRL/150003**
Name of Product:	***Lactobacillus brevis* TAK 124-1 NCIMB 42149**
Active Agent (s):	***Lactobacillus brevis* TAK 124-1 NCIMB 42149**
Rapporteur Laboratory:	**Centro di Referenza Nazionale per la Sorveglianza ed il Controllo degli Alimenti per Animali (C.Re.A.A), Torino, Italy**
Report prepared by:	**Stefania Squadrone**
Report checked by: Date:	**Rebeca Fernandez Orozco & Zigmas Ezerskis (EURL-FA)** **26/10/2015**
Report approved by: Date:	**Christoph von Holst** **27/10/2015**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4(1) for *Lactobacillus brevis TAK 124-1 NCIMB 42149* 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* for all animal species. The *feed additive* is to be marketed as a powder containing a minimum concentration of $1.0x10^{11}$ colony forming units (CFU) /g *Lactobacillus brevis TAK 124-1 NCIMB 42149*. The original strain is deposited at NCIMB Ltd. (National Collection of Industrial, Food and Marine Bacteria, Scotland). The *feed additive* is intended to be added to *silage* via a water suspension at a minimum dose of $1.0x10^{8}$ CFU/kg fresh *silage*.

For the identification of *Lactobacillus brevis TAK 124-1 NCIMB 42149*, the Applicant submitted the carbohydrate fermentation patterns (API system) and molecular methods: Internal Transcribed Spacer Polymerase Chain Reaction (ITS-PCR) and Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR). However, the EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised standard methodology for microbial identification.

For enumeration of *Lactobacillus brevis TAK 124-1 NCIMB 42149* in *feed additive*, the Applicant submitted a pour plate method based on the ring-trial validated CEN method (EN 15787). Based on the performance characteristics available the EURL recommends for official control the CEN method for the enumeration of *Lactobacillus brevis TAK 124-1 NCIMB 42149* in the *feed additive*.

Since the accurate quantification of *Lactobacillus brevis TAK 124-1 NCIMB 42149* added to *silage* is not experimentally achievable, the Applicant did not provide any experimental method or data. Therefore, 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 brevis TAK 124-1 NCIMB 42149, 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 brevis TAK 124-1 NCIMB 42149* under the category / functional group 1(k) "technological additives" / "silage additives", according to Annex I of Regulation (EC) No 1831/2003 [1]. Specifically, authorisation is sought for the use of the *feed additive* for all animal species [1,2].

The *feed additive* is to be marketed as a powder containing a minimum concentration of $1.0x10^{11}$ colony forming units (CFU) /g *Lactobacillus brevis TAK 124-1 NCIMB 42149*. The original strain is deposited at NCIMB Ltd. (National Collection of Industrial, Food and Marine Bacteria, Scotland [3].

The *feed additive* is intended to be added to *silage* via a water suspension at a minimum dose of $1.0x10^{8}$ CFU/kg fresh *silage* [2,4].

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 brevis TAK 124-1 NCIMB 42149* 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 the identification of the strain of *Lactobacillus brevis TAK 124-1 NCIMB 42149*, the Applicant used the carbohydrate fermentation patterns (API system), ITS -PCR (Internal Transcribed Spacer Polymerase Chain Reaction) and ERIC-PCR (Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction), and confirmed the identification of the strain by sequence analysis of 16S rRNA [5]. However, the EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised standard methodology for genetic identification [6].

Qualitative and quantitative composition of impurities in the additive

The Applicant analysed the *feed additive* for microbial contaminants (e.g. *Salmonella*, *Enterobacteria*, yeast and moulds) by using appropriate EN ISO standards [7]. For undesirable substances (i.e. arsenic, cadmium, lead, mercury, mycotoxins) several internationally recognised standard methods are available at the respective European Union Reference Laboratories [8].

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

For the enumeration of *Lactobacillus brevis TAK 124-1 NCIMB 42149* in *feed additive* the Applicant submitted pour plate method [9] based on the ring-trial validated CEN method (EN 15787) [10].

According to the EN 15787 method, 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 at 37°C for 48 to 72 hours and must be placed in anaerobiosis for enumeration of lactobacilli. The performance characteristics of the EN 15787 method reported after logarithmic transformation of measured values (CFU) are [10]:

- a repeatability standard deviation (S_r) of 0.24 $\log_{10}$ CFU/g;
- a reproducibility standard deviation (S_R) ranging from of 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 performance characteristics available the EURL recommends for official control the EN 15787 method for the enumeration of *Lactobacillus brevis TAK 124-1 NCIMB 42149* in the *feed additive*.

Since the accurate quantification of *Lactobacillus brevis TAK 124-1 NCIMB 42149* added to *silage* is not experimentally achievable, the Applicant did not provide any experimental method or data. Therefore, 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.

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 brevis TAK 124-1 NCIMB 42149* and the ring-trial validated pour plate EN 15787 method for the enumeration the active substance in the *feed additive*.

Since the accurate quantification of *Lactobacillus brevis TAK 124-1 NCIMB 42149* added to *silage* is not experimentally achievable, the Applicant did not provide any experimental method or data. Therefore, the EURL cannot evaluate or recommend any method for official control to quantify the active substance in *silage*.

Recommended text for the register entry (analytical method)

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

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Lactobacillus brevis TAK 124-1 NCIMB 42149* 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/G1: Forw. Appl. 1831/0012-2015

[2] *Application, Proposal for Register Entry, Annex A

[3] *Technical dossier, Section II – Identity, characterisation of the active substance

[4] *Technical dossier, Section II.2.5 – Conditions of use of the additive

[5] *Technical dossier, Section II.2.2 – Identification and molecular characteristics

[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 – Annex II.4

[8] 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

[9] *Technical dossier, Section II. 2.6 – Method of analysis and reference samples

[10] EN 15787:2009 – Animal feedingstuffs - 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-2015-0014

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was Centro di Referenza Nazionale per la Sorveglianza ed il Controllo degli Alimenti per Animali (C.Re.A.A). 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

The following National Reference Laboratories contributed to this report:

- Laboratoire de Rennes (SCL L35), Service Commun des Laboratoires DGCCRF et DGDDI, Rennes (FR)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Univerza v Ljubljani. Veterinarska fakulteta. Nacionalni veterinarski inštitut. Enota za patologijo prehrane in higieno okolja, Ljubljana (SI)