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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**

B-Act[®]

(FAD-2015-0016; CRL/140039)



**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-2015-0016 - CRL/140039**

Name of Product: **B-Act[®]**

Active Agent (s): ***Bacillus licheniformis* BL 11 (DSM 28710)**

Rapporteur Laboratory: **Centre wallon de Recherches
agronomiques (CRA-W), Gembloux,
Belgium**

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Date: **22/10/2015**

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Date: **22/10/2015**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4(1) for *B-Act*[®] under the category / functional group 4(b) 'zootechnical additives' / 'gut flora stabilisers', according to Annex I of Regulation (EC) No 1831/2003. Specifically, authorization is sought for the use of the *feed additive* for chickens for fattening and chickens reared for laying.

According to the Applicant, the *feed additive* contains as active substance viable spores of the non-genetically modified strain *Bacillus licheniformis* BL 11 (DSM 28710). The *feed additive* is to be marketed as a powder containing a minimum *Bacillus licheniformis* BL 11 (DSM 28710) of 3.2×10^9 Colony Forming Unit (CFU)/g. The *feed additive* is intended to be used directly in *feedingstuffs* or through *premixtures* at a minimum dose of 1.6×10^9 CFU /kg complete *feedingstuffs*.

For the identification of *Bacillus licheniformis* BL 11 (DSM 28710) the EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised standard methodology for genetic identification. This standard methodology for microbial identification is currently being evaluated by the CEN Technical Committee 327 to become a European Standard.

For enumeration of *Bacillus licheniformis* BL 11 (DSM 28710) in *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted the ring-trial validated spread plate CEN method EN 15784 which was already evaluated by EURL in the frame of previous *Bacillus licheniformis* dossiers. Based on the performance characteristics available the EURL recommends for official control the EN 15784 method for the enumeration of *Bacillus licheniformis* BL 11 (DSM 28710) in the *feed additive*, *premixtures* and *feedingstuffs*.

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

Bacillus licheniformis BL 11 (DSM 28710), technological additives, gut flora stabilisers, chickens for fattening, chickens reared for laying.

1. BACKGROUND

In the current application authorisation is sought under Article 4(1) for *B-Act*[®] under the category / functional group 4(b) 'zootechnical additives' / 'gut flora stabilisers', according to Annex I of Regulation (EC) No 1831/2003 [1]. Specifically, authorization is sought for the use of the *feed additive* for chickens for fattening and chickens reared for laying [1,2].

According to the Applicant, the *feed additive* contains as active substance viable spores of the non-genetically modified strain *Bacillus licheniformis* BL 11 [2, 3]. The strain is deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) with the deposit number DSM 28710 [3].

The *feed additive* is to be marketed as a powder containing a minimum *Bacillus licheniformis* BL 11 (DSM 28710) concentration of 3.2×10^9 Colony Forming Unit (CFU) / g [3].

The *feed additive* is intended to be used directly in *feedingstuffs* or through *premixtures* at a minimum dose of 1.6×10^9 CFU / kg complete *feedingstuffs* [2,3].

Note: The EURL previously evaluated the analytical methods for the determination of *Bacillus licheniformis* in the frame of several dossiers (e.g. FAD-2009-0059; FAD-2013-0017) [4].

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009, 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 *B-Act*[®] and their suitability to be used for official controls in the frame of the authorisation were evaluated.

3. EVALUATION

Identification /Characterisation of added feed additive

For the identification of *Bacillus licheniformis* BL 11 (DSM 28710), the Applicant used partial 16S rRNA gene sequence analysis [3]. The EURL recommends instead for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised standard methodology for genetic identification [5]. This standard methodology for microbial

identification is currently being evaluated by the CEN Technical Committee 327 to become a European Standard.

Qualitative and quantitative composition of impurities in the additive

The Applicant analysed the *feed additive* for Salmonella by using the method described in the technical dossier [3]. As for the determination of other undesirable substances in the *feed additive* (e.g. arsenic, cadmium, lead, mercury, mycotoxins and dioxins) several internationally recognised standard methods are available at the respective European Union Reference Laboratories [6].

Description of the analytical methods for the determination of the active substances in feed additive, premixtures and feedingstuffs

For enumeration of *Bacillus licheniformis* BL 11 (DSM 28710) in *feed additive, premixtures* and *feedingstuffs* the Applicant submitted the ring-trial validated spread plate CEN method EN 15784 [3,7] that was already evaluated by EURL in the frame of previous *Bacillus licheniformis* dossiers [4].

Twenty grams of the *feed additive* (or 50g of *premixtures* or *feedingstuffs*) are suspended in a phosphate buffered saline (or in 0.2% sodium hydroxide solution for *premixtures* or *feedingstuffs*). From this, one new dilution is prepared and heat-treated at 80 °C for 10 minutes. Decimal dilutions are prepared from the heat treated suspension, spread plated on tryptone soya agar and incubated at 37 °C for 16-24 h aerobically. The performance characteristics of the method reported after logarithmic transformation are [7]:

- *repeatability* standard deviation (S_r) ranging from 0.07 to 0.09 \log_{10} CFU/g;
- *reproducibility* standard deviation (S_R) ranging from of 0.32 to 0.35 \log_{10} CFU/g; and
- limit of quantification (LOQ) of 3×10^7 CFU/kg of *feedingstuffs*, well below the minimum dose of 1.6×10^9 CFU/kg *feedingstuffs* proposed by the Applicant.

Note: The EN 15784 method is not applicable to mineral feeds composed mainly of minerals and containing at least 40 % crude ash. For these matrices laboratories may consider using the ring-trial validated VDLUFA method 28.2.2 instead [8].

Based on the performance characteristics available, the EURL recommends for official control the ring-trial validated EN 15784 method for the enumeration of *Bacillus licheniformis* BL 11 (DSM 28710) in *feed additive, premixtures* and *feedingstuffs*.

Note: The EN 15784 method is not applicable to mineral feeds composed mainly of minerals and containing at least 40 % crude ash. For these matrices laboratories may consider using the ring-trial validated VDLUFA method 28.2.2 instead [9].

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 *Bacillus licheniformis* BL 11 (DSM 28710) and the ring-trial validated spread plate method EN 15784 for the enumeration of the strain in the *feed additive, premixtures* and *feedingstuffs*.

Note: The EN 15784 method is not applicable to mineral feeds composed mainly of minerals and containing at least 40 % crude ash. For these matrices laboratories may consider using the ring-trial validated VDLUFA method 28.2.2 instead [8].

Recommended text for the register entry (analytical method)

- Identification: Pulsed Field Gel Electrophoresis (PFGE)
- Enumeration in the *feed additive, premixtures* and *feedingstuffs*: spread plate method following heat treatment - EN 15784

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *B-Act®* 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: F.A. 1831/0016-2015
- [2] *Application, Proposal for Register Entry, Annex A
- [3] *Technical dossier, Section II, Identity, characterisation and conditions of use of the additive
- [4] EURL Evaluation Reports:
<https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2009-0059.pdf>
<https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2013-0017.pdf>
- [5] 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)
- [6] 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

[7] EN 15784:2009 - Animal feeding stuffs - Isolation and enumeration of presumptive *Bacillus* spp.

[8] VDLUFA method – Enumeration of *Bacillus licheniformis* and *Bacillus subtilis* (VDLUFA Methodenbuch Bd.III, 28.2.2)

*Refers to Dossier no: FAD-2015-0016

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was Centre wallon de Recherches agronomiques (CRA-W), Gembloux, 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

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- Thüringer Landesanstalt für Landwirtschaft (TLL). Abteilung Untersuchungswesen. Jena (DE)
- Laboratoire de Rennes (SCL L35), Service Commun des Laboratoires DGCCRF et DGDDI, Rennes (FR)
- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)