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JRC F.5/CvH/SB/AS/Ares

**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**

**Lentilactobacillus buchneri BioCC 203 DSM 32650**  
*(FEED-2021-0246; CRL/210042)*





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in connection with the Application for Authorisation of a  
Feed Additive according to Regulation (EC) No 1831/2003**

Dossier related to: **FEED-2021-0246 - CRL/210042**

Name of Product: ***Lentilactobacillus buchneri BioCC 203  
DSM 32650***

Active Agent (s): **Lentilactobacillus buchneri**

Rapporteur Laboratory: **European Union Reference Laboratory for  
Feed Additives (EURL-FA)  
JRC Geel, Belgium**

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Date: **02/12/2022**

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Date: **02/12/2022**

## EXECUTIVE SUMMARY

In the current application an authorisation is sought under Article 4 for a preparation of *Lentilactobacillus buchneri* BioCC 203 DSM 32650 under the category / functional group 1(k) 'technological additives' / 'silage additives', according to Annex I of Regulation (EC) No 1831/2003. The 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 *Lentilactobacillus buchneri* BioCC 203 DSM 32650. The *feed additive* is to be marketed as a preparation with a minimum content of  $1.0 \times 10^{10}$  Colony Forming Unit (CFU) / g product. The *feed additive*, after *water* addition, is intended to be sprayed homogeneously directly on fresh forage. The Applicant proposed a minimum dosage of the *active substance* of  $1.0 \times 10^5$  CFU / g fresh forage, corresponding to 1.0 g of product to be distributed on one ton of fresh forage.

For the enumeration of *Lentilactobacillus buchneri* BioCC 203 DSM 32650 in the *feed additive* and *water* the Applicant presented for official control the ring-trial validated spread plate (or pour plate) method EN 15787.

Based on the performance characteristics, as already concluded for similar Lactoplantibacilli reports, the EURL recommends for official control the ring-trial validated EN 15787 method for the enumeration of *Lentilactobacillus buchneri* BioCC 203 DSM 32650 in the *feed additive* and *water*.

Furthermore, for the identification of *Lentilactobacillus buchneri* BioCC 203 DSM 32650, the Applicant proposed Enterobacterial Repetitive Intergenic Consensus - Polymerase Chain Reactions (ERIC-PCR). In former reports for similar dossiers, the EURL recommended for official control DNA sequencing methods or Pulsed-Field Gel Electrophoresis (PFGE). The EURL considers that all the above-mentioned methodologies are fit-for-purpose for the bacterial identification of authorised additives at a strain level.

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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

## KEYWORDS

*Lentilactobacillus buchneri* BioCC 203 DSM 32650, technological additive, silage additive, all animal species.

## 1. BACKGROUND

In the current application an authorisation is sought under Article 4(1) (new feed additive) for a preparation of *Lentilactobacillus buchneri* BioCC 203 DSM 32650 under the category / functional group 1(k) 'technological additives' / 'silage additives', according to Annex I of Regulation (EC) No 1831/2003 [1-3]. The authorisation is sought for the use of the *feed additive* for all animal species [2].

The *feed additive* is intended to be marketed as a dried powder preparation containing as active substance *Lentilactobacillus buchneri* BioCC 203 DSM 32650 [4,5]. The preparation contains viable cells of the microorganism with a minimum content of  $1.0 \times 10^{11}$  Colony Forming Unit (CFU) / g product [5].

The *Lentilactobacillus buchneri* BioCC 203 DSM 32650 is a non-genetically modified strain. The microorganism is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) [6].

The *feed additive*, after *water* addition, is intended to be sprayed homogeneously directly on fresh forage [7]. The Applicant proposed a minimum dosage of the *active substance* of  $1.0 \times 10^5$  CFU/g fresh forage, corresponding to 1.0 g of product to be distributed on one ton of fresh forage [7].

## 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 *Lentilactobacillus buchneri* and their suitability to be used for official controls in the frame of the authorisation were evaluated.

## 3. EVALUATION

***Description of the analytical methods for the determination of the active substance in the feed additive, premixtures, feedingstuffs and when appropriate water (section 2.6.1 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)***

For the enumeration of *Lentilactobacillus buchneri* BioCC 203 DSM 32650 in the *feed additive* and *water* the Applicant proposed the ring-trial validated EN 15787:2009 method [8]. The method has been recently revised by CEN resulting in updated method dedicated for the enumeration of lactobacilli spp. in *feedingstuffs* (additives, *premixtures* and compound feeds

excluding mineral feeds) that contain lactobacilli as a single microorganism component or in a mixture with other microorganisms (EN 15787:2021) [9].

Following the updated method's protocol, the sample (5 to 50 g) is suspended in phosphate buffered saline containing Polysorbate 80 (Tween® 80) (tPBS). For serial dilutions, the peptone salt solution (PSS) is used. The appropriate dilutions are then mixed on Petri plates using spread plate (or pour plate) methods with MRS (de Man, Rogosa, Sharp) agar. Alternatively, the MRS agar can be acidified or include triphenyl tetrazolium chloride (TTC). However, for routine purposes the non-modified MRS agar is an appropriate medium. The agar plates are incubated anaerobically at 37 °C for 48 to 72 h [9].

The following performance characteristics, expressed in terms of precision, are reported in the frame of the ring-trial validation studies after logarithmic transformation of the CFU values ranging from 7.40 to 8.03 log<sub>10</sub> CFU/g: a standard deviation for *repeatability* (S<sub>r</sub>) ranging from 0.10 to 0.26 log<sub>10</sub> CFU/g and a standard deviation for *reproducibility* (S<sub>R</sub>) ranging from 0.18 to 0.39 log<sub>10</sub> CFU/g [9].

In addition, a limit of quantification (LOQ) of 3x10<sup>3</sup> CFU/g can be derived using the considerations of EN ISO 7218 standard [10].

Furthermore, in the frame of stability studies, the Applicant successfully performed experiments demonstrating the suitability of the CEN method in the *feed additive* preparation [11,12].

Based on the performance characteristics and the available information, the EURL recommends for official control the ring-trial validated EN 15787 method for the enumeration of *Lentilactobacillus buchneri* BioCC 203 DSM 32650 in the *feed additive* and *water*.

***Methods of analysis for the determination of the residues of the additive in food (section 2.6.2 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)***

An evaluation of corresponding methods of analysis is not relevant for the present application.

***Identification/Characterisation of the feed additive (section 2.6.3 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)***

For the taxonomic identification of *Lentilactobacillus buchneri* BioCC 203 DSM 32650, the Applicant proposed Enterobacterial Repetitive Intergenic Consensus - Polymerase Chain Reactions (ERIC-PCR) [8,13].

In former reports for similar dossiers, the EURL recommended for official control DNA sequencing methods or Pulsed-Field Gel Electrophoresis (PFGE). PFGE is a generally recognised methodology for the genetic identification of bacterial strains [14]. The method has been recently ring trial validated and is supposed to become a CEN Technical specification [15].

The EURL considers that all the above-mentioned methodologies are fit-for-purpose for the bacterial identification of authorised additives at a strain level.

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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

#### **4. CONCLUSIONS AND RECOMMENDATIONS**

In the frame of this authorisation the EURL recommends for official control (i) Enterobacterial Repetitive Intergenic Consensus - Polymerase Chain Reactions (ERIC-PCR) or DNA sequencing methods or Pulsed-Field Gel Electrophoresis (PFGE) for the identification of *Lentilactobacillus buchneri* BioCC 203 DSM 32650; and (ii) the ring-trial validated spread plate (or pour plate) CEN method (EN 15787) for the enumeration of *Lentilactobacillus buchneri* BioCC 203 DSM 32650 in the *feed additive* and *water*.

##### ***Recommended text for the register entry (analytical method)***

- Identification: Enterobacterial Repetitive Intergenic Consensus - Polymerase Chain Reactions (ERIC-PCR) or DNA sequencing methods or Pulsed-Field Gel Electrophoresis (PFGE)
- Enumeration in the *feed additive* and *water*: Spread plate (or pour 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 *Lentilactobacillus buchneri* BioCC 203 DSM 32650 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] Forwarding of applications for authorisation of feed additives in accordance with Regulation (EC) No 1831/2003 – E-Submission Food Chain platform – <https://webgate.ec.europa.eu/esfc/#/applications/306>  
<https://open.efsa.europa.eu/questions/EFSA-Q-2021-00381>
- [2] \*Application, Application form\_Annex I
- [3] \*Scientific Summary, Section I: 1.2.2.1 Identity of the technological additive
- [4] \*Scientific Summary, Section I: 1.2.2.1.3 Qualitative and quantitative composition (active substance, other components, impurities, batch to batch variation)

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- [5] \*Scientific Summary, Section I: 1.2.2.3.2 Additive
- [6] \*Technical dossier, Section II: II.2.2 Characterisation of the active substance(s)/agent(s)
- [7] \*Technical dossier, Section II: Sect\_II\_Identity\_2.5\_L.buchneri BioCC 203 DSM 32650
- [8] \*Technical dossier, Section II: 2.6 Methods of analysis and reference samples
- [9] EN 15787:2021 – Animal feeding stuffs: Methods of sampling and analysis - Detection and enumeration of *Lactobacillus* spp. used as feed additive
- [10] ISO 7218:2007 – Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations
- [11] \*Scientific Summary, Section I: 1.2.2.4.1 Stability
- [12] \*Technical dossier, Section II: Annex\_II\_33\_Shelf life-1
- [13] \*Annex\_II\_12\_Plasmid profiling of and genetic stability-1
- [14] EURL reports: [https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives/eurl-fa-authorisation/eurl-fa-evaluation-reports\\_en](https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives/eurl-fa-authorisation/eurl-fa-evaluation-reports_en)
- [15] CEN project TC 327 WI00327127 (2020): DNA fingerprinting of lactobacilli, pediococci, enterococci and bacilli in animal feeds by pulsed field gel electrophoresis (PFGE) Draft Report of a validation trial
- \*Refers to Dossier no: FEED-2021-2246

## 7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation is the European Union Reference Laboratory for Feed Additives, JRC, 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 (EU) 2015/1761.

## 8. ACKNOWLEDGEMENTS

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- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, Pesca, Alimentació i Medi Natural. Generalitat de Catalunya, Cabrils (ES)
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)