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European Union Reference Laboratory
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

Lentilactobacillus buchneri BioCC 228 DSM 32651

(FEED-2023-16653; CRL/230027)

European Union Reference Laboratory
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Dossier related to:	**FEED-2023-16653 - CRL/230027**
Name of Product :	***Lentilactobacillus buchneri BioCC 228 DSM 32651***
Active Agent (s):	**Lentilactobacillus buchneri *BioCC 228 DSM 32651***
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **JRC Geel, Belgium**
Report prepared by:	**María José González de la Huebra**
Report checked by: Date:	**Zigmas Ezerskis** **10/11/2023**
Report approved by: Date:	**Christoph von Holst** **10/11/2023**

EXECUTIVE SUMMARY

In the current application an authorisation is sought under Article 4(1) for *Lentilactobacillus buchneri BioCC 228* DSM 32651 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 the non-genetically modified *Lentilactobacillus buchneri BioCC 228* DSM 32651 as an *active substance* at a minimum concentration of 1 x 10^{11} Colony Forming Unit (CFU) / g. The *feed additive* is intended to be added to the forage, used for ensiling process at a recommended dosage of 1 x 10^{5} CFU / g fresh *silage*.

For the identification of *Lentilactobacillus buchneri BioCC 228* DSM 32651, the Applicant proposed Enterobacterial Repetitive Intergenic Consensus - Polymerase Chain Reactions (ERIC-PCR) and Pulsed-Field Gel Electrophoresis (PFGE). In former reports for similar dossiers, the EURL recommended for official control DNA sequencing methods or Pulsed-Field Gel Electrophoresis (PFGE) described in CEN Technical Specification (CEN/TS 17697). The EURL considers that all the above-mentioned methodologies are suitable for official control for the bacterial identification of *Lentilactobacillus buchneri BioCC 228* DSM 32651 at strain level.

For the enumeration of *Lentilactobacillus buchneri BioCC 228* DSM 32651 in the *feed additive* the Applicant proposed the ring-trial validated EN 15787 method. Based on the available information, the EURL recommends for official control the ring-trial validated EN 15787 method for the enumeration of *Lentilactobacillus buchneri BioCC 228* DSM 32651 in the *feed additive.*

As the unambiguous determination of *Lentilactobacillus buchneri BioCC 228* DSM 32651 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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

KEYWORDS

Lentilactobacillus buchneri BioCC 228 DSM 32651, technological additives, silage additives, all animal species.

1. BACKGROUND

In the current application an authorisation is sought under Article 4(1) (new feed additive) for *Lentilactobacillus buchneri BioCC 228* DSM 32651 under the category / functional group 1(k) 'technological additives' / 'silage additives', according to Annex I of Regulation (EC) No 1831/2003 [1-2]. The authorisation is sought for the use of the *feed additive* for all animal species [1-2].

According to the Applicant, the *feed additive* contains *Lentilactobacillus buchneri BioCC 228* DSM 32651 as an *active substance* at a minimum concentration of 1 x 10^{11} Colony Forming Unit (CFU) / g [3].

The Applicant stated that the *Lentilactobacillus buchneri BioCC 228* DSM 32651 is a non-genetically modified strain [4]. The microorganism is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) [3].

The *feed additive* is intended to be added to the forage, used for ensiling process at a recommended dosage of 1 x 10^5 CFU / g fresh *silage* [5].

Note: The EURL has previously evaluated the analytical methods for the determination of another *Lentilactobacillus buchneri* strain in the frame of a previous dossier [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 *Lentilactobacillus buchneri BioCC 228* DSM 32651 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, compound feed 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 228* DSM 32651 in the *feed additive* the Applicant proposed the ring-trial validated EN 15787:2009 method [7]. However, the EURL is aware that this method has been 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) [8].

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 [8].

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 $\log_{10}$ CFU/g: a standard deviation for *repeatability* (S_r) ranging from 0.10 to 0.26 $\log_{10}$ CFU/g and a standard deviation for *reproducibility* (S_R) ranging from 0.18 to 0.39 $\log_{10}$ CFU/g [8].

In addition, a limit of quantification (LOQ) of $3x10^3$ CFU/g can be derived using the considerations of EN ISO 7218 standard [9].

The Applicant demonstrated the fitness-for-purpose of the proposed method by providing experimental data obtained for the *feed additive* in the frame of the batch-to-batch variation [3] and in the stability studies [10].

Based on the available information, the EURL recommends for official control the ring-trial validated EN 15787 method for the enumeration of *Lentilactobacillus buchneri BioCC 228* DSM 32651 in the *feed additive.*

As the unambiguous determination of *Lentilactobacillus buchneri BioCC 228* DSM 32651 added to silage is not achievable by analysis, the EURL cannot evaluate nor recommend the EN 15787 or any other method for official control to enumerate *Lentilactobacillus buchneri BioCC 228* DSM 32651 in *silage*.

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 identification of *Lentilactobacillus buchneri BioCC 228* DSM 32651, the Applicant proposed Enterobacterial Repetitive Intergenic Consensus - Polymerase Chain Reactions (ERIC-PCR) and Pulsed-Field Gel Electrophoresis (PFGE) [7].

In former reports for similar dossiers, the EURL recommended for official control DNA sequencing methods or Pulsed-Field Gel Electrophoresis (PFGE), a generally recognised methodology for the genetic identification of bacterial strains. The method has been ring-trial validated and recently published as a CEN Technical Specification CEN/TS 17697 [11].

The EURL considers that that all the above-mentioned methodologies are suitable for official control for the bacterial identification of *Lentilactobacillus buchneri BioCC 228* DSM 32651 at 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) CEN Technical Specification (CEN/TS 17697) for the identification of *Lentilactobacillus buchneri BioCC 228* DSM 32651; and ii) the ring-trial validated spread plate (or pour plate) CEN method (EN 15787) for the enumeration of *Lentilactobacillus buchneri BioCC 228* DSM 32651 in the *feed additive*.

As the unambiguous determination of *Lentilactobacillus buchneri BioCC 228* DSM 32651 added to silage is not achievable by analysis, the EURL cannot recommend the EN 15787 or any other method for official control to enumerate *Lentilactobacillus buchneri BioCC 228* DSM 32651 in *silage*.

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) (CEN/TS 17697)
- Enumeration in the *feed additive*: 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 228* DSM 32651 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/45934 https://open.efsa.europa.eu/questions/EFSA-Q-2023-00392

[2] *Application, Annex 1

[3] *Technical dossier, Sect_II_Identity_2.1_2.2_L.buchneri BioCC 228 DSM 32651_Conf

[4] *Technical dossier, Sect_V_Post_Mark_L. buchneri BioCC 228 DSM 32651

[5] *Technical dossier, Section II: 2.5 Conditions of use of the additive

[6] EURL report: https://joint-research-centre.ec.europa.eu/document/download/66f50598-7cbb-4bf6-92ed-5e7131049b48_en?filename=finrep-feed-2021-0246-lentilactobacillus_buchneriBioCC.pdf

[7] *Technical dossier, Sect_II_Identity_2.6_L.buchneri BioCC 228 DSM 32651

[8] EN 15787:2021 – Animal feeding stuffs: Methods of sampling and analysis - Detection and enumeration of Lactobacillus spp. used as feed additive

[9] ISO 7218:2007 – Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations

[10] *Technical dossier, Sect_II_Identity_2.4_L.buchneri BioCC 228 DSM 32651

[11] CEN/TS 17697:2023 - Animal feeding stuffs - Methods of sampling and analysis - PFGE typing of Lactobacilli, Pediococci, Enterococci and Bacilli in animal feeds

*Refers to Dossier no: FEED-2023-16653

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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- Państwowy Instytut Weterynaryjny, Pulawy (PL)
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