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

Dossier related to:	FAD-2010-0032 EURL/090041
Product Name:	Biomax
Active Substance(s):	*Lactoccocus lactis DSM 11037*
Rapporteur Laboratory:	European Union Reference Laboratory for Feed Additives (EURL-FA)
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Report approved by: **Date:**	Christoph von Holst 25/01//2011

EXECUTIVE SUMMARY

In the current application authorisation is sought for the microbial feed additive *Lactoccocus lactis DSM 11037* under the category 'technological additives', functional group 'silage additives' according to Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the *feed additive* to be placed on the market in the form of powder containing minimum concentration of $1x10^5$ CFU/g of *Lactoccocus lactis DSM 11037*. The intended use of the current application is for all animal species up to slaughter age. It is proposed to be mixed into silage with water providing a minimum concentration in silage of $1x10^5$ CFU/kg.

For the enumeration of *Lactoccocus lactis DSM 11037*, in *feed additive*, the Applicant proposes a pour plate method based on ISO 15214, using de Man, Rogosa and Sharpe agar (MRS) at pH 5.7. A limit of detection (LOD) of 10^5 CFU/kg is reported in the ISO 7218 standard.

The Applicant did not provide any experimental method or data for the determination of *Lactoccocus lactis DSM 11037* in *silage*. Furthermore, the unambiguous determination of the content of *Lactoccocus lactis DSM 11037* added to silage is not achievable by analysis. Therefore the EURL cannot evaluate nor recommend any method for official control to determine *Lactoccocus lactis DSM 11037* in *silage*.

Molecular methods were used by the Applicant for identification of 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

Lactoccocus lactis DSM 11037, technological additives, silage additives.

1. BACKGROUND

Lactoccocus lactis DSM 11037 is a feed additive for which authorisation under Article 4(1) is sought under the category of 'technological additives' functional group 'silage additives' according to Annex I of Regulation (EC) No 1831/2003 [1]. Specifically, authorisation is sought for the *feed additive* to be placed on the market in the form of powder, containing

minimum concentration of $1x10^5$ CFU/g of *Lactoccocus lactis DSM 11037* [2]. *Lactoccocus lactis DSM 11037* is an improved strain of a dairy starter culture strain. The original strain has been used extensively in the dairy industry around the world for many years and is deposited in Deutsche Sammlung von Mikro-organismem und Zelkulturen (DSMZ) [3]. The intended use of the current application is for all animal species up to slaughter age. It is proposed to be mixed into silage with water providing a minimum concentration of $1x10^5$ CFU/kg [4].

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 authorizations 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 *Lactoccocus lactis DSM 11037* 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 characterization of the strain *Lactoccocus lactis DSM 11037* the Applicant used Dot Blot hybridization with subspecies specific oligonucleotide probes (16S ribosomal RNA) and API characterization [5]. This method is suitable for the purpose of analysis. However, the EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised standard methodology for microbial identification [6].

Qualitative and quantitative composition of any impurities in the additive

The Applicant analysed the *feed additive* for microbial contaminants (such as Enterobacteria, *Escherichia coli*, Salmonella spp. and yeasts) by using appropriate EN ISO tests [7]. 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 agent(s) in feed additive, premixtures and feedingstuffs

For the enumeration of *Lactoccocus lactis DSM 11037*, in *feed additive*, the Applicant proposes a pour plate method based on ISO 15214, using de Man, Rogosa and Sharpe agar (MRS) at pH 5.7. The sample is dissolved in boiled water and 1ml of the first decimal dilution is transferred into sterile Petri dishes. Approximately 15 ml of MRS agar is poured into Petri dishes, cooled to 47 °C. The inoculum is carefully mixed with the medium and the mixture allowed to solidify. The dishes are inverted and incubated at 30°C for 72 h ± 3h. [8]. A limit of detection (LOD) of 10^5 CFU/kg is reported in the ISO 7218 standard [9].

The EURL recommends for official control the ISO method "ISO 15214" for the determination of *Lactoccocus lactis DSM 11037* in the *feed additive per se*.

The Applicant did not provide any experimental method or data for the determination of *Lactoccocus lactis DSM 11037* in *silage*. Furthermore, the unambiguous determination of the content of *Lactoccocus lactis DSM 11037* added to silage is not achievable by analysis. Therefore the EURL cannot evaluate nor recommend any method for official control to determine *Lactoccocus lactis DSM 11037* 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 the ISO method -"ISO 15214"- for the enumeration of the active agent *Lactoccocus lactis DSM 11037* in the *feed additive*.

For the analysis of the identity of the bacterial strain, *Lactoccocus lactis DSM 11037,* the EURL recommends Pulsed Field Gel Electrophoresis (PFGE) for official control.

The Applicant did not provide any experimental method or data for the determination of *Lactoccocus lactis DSM 11037* in *silage*. Furthermore, the unambiguous determination of the content of *Lactoccocus lactis DSM 11037* added to silage is not achievable by analysis. Therefore the EURL cannot evaluate nor recommend any method for official control to determine *Lactoccocus lactis DSM 11037* in *silage*.

Recommended text for the register entry, fourth column (Composition, chemical formula, description, analytical method)

- Enumeration: Pour plate method de Man, Rogosa and Sharpe agar (ISO 15214)
- Identification: 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 *Lactoccocus lactis DSM 11037,* for all animal species up to slaughter age, have been sent to the European Union Reference Laboratory for Feed Additives Authorisation. The dossier has been made available to the EURL by EFSA.

6. REFERENCES

[1] *Application/Ref: SANCO/D/2: Forw.Appl.1831/0024-2010

[2] *Application, Annex A, Proposal for register entry

[3] *Technical Dossier, Section II.2.1. Identity of the additive

[4] *Technical Dossier, Section II 2.5.1 Conditions of use of the additive

[5] *Technical Dossier, Annex II.6

[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 2.6.3. Methods of the analysis relating to the identity and characterisation of the additive

[8] *Technical Dossier, Section II 2.6 Methods of analysis and reference samples

[9] ISO 7218:1996, Microbiology of food and animal feedingstuffs – General rules for microbiological examinations

*Refers to Dossier no: FAD-2010-0032

7. RAPPORTEUR LABORATORY

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.

8. ACKNOWLEDGEMENTS

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