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

Dossier related to: **FAD-2010-0150 - CRL/100157**
FAD-2012-0001 - CRL/110025

Name of Feed Additive: **Oralin**

Active Substance(s): ***Enterococcus faecium***
DSM 10663/NCIMB 10415 (E 1707)

Rapporteur Laboratory: **European Union Reference Laboratory
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Date: **12/10/2012**

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Date: **16/10/2012**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 10(2)¹ and 4(1)² for *Enterococcus faecium* DSM 10663/NCIMB 10415 (E 1705), under the category/functional group 4(b), "zootechnical additives/gut flora stabilisers", according to the classification system of Annex I of Regulation (EC) No 1831/2003. The *feed additive* will be marketed in several forms (i.e. powder, granulated, coated and liquid) with *Enterococcus faecium* DSM 10663/NCIMB 10415 concentrations ranging from 1×10^{10} to 3.5×10^{10} CFU/g. Specifically, authorisation is sought for the use of the *feed additive* for chickens for fattening¹, calves for rearing¹, piglets (suckling and weaned)¹, turkeys for fattening¹, dogs¹ and cats². The *feed additive* is intended to be used either via *premixtures* or incorporated directly into the *feedingstuffs*. The Applicant proposed *Enterococcus faecium* DSM 10663/NCIMB 10415 content in complete *feedingstuffs* ranging from 1×10^7 to 3.5×10^{10} CFU/kg, depending on the animal species.

For the enumeration of *Enterococcus faecium* DSM 10663/NCIMB 10415 in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant proposed a ring-trial validated double-layer plate method, using Slanetz and Bartley agar (VDLUFA 28.2.3). The EURL identified instead the internationally recognised ring-trial validated spread plate CEN method (EN 15788), using Bile Esculin Azide Agar, for the enumeration of *Enterococcus* spp. The performance characteristics of the CEN method reported after logarithmic transformation (CFU) are:

- a repeatability standard deviation (s_r) ranging from 0.12 to 0.2 \log_{10} CFU/g,
- a reproducibility standard deviation (s_R) ranging from 0.23 to 0.41 \log_{10} CFU/g; and
- a limit of detection (LOD) of 1×10^5 CFU/kg *feedingstuffs*.

Based on the performance characteristics presented, the EURL recommends for official control, the CEN method (EN 15788) for the enumeration of *Enterococcus faecium* DSM 10663/NCIMB 10415 in *feed additive*, *premixtures* and *feedingstuffs*.

Morphology study and API characterisation were used by the Applicant to identify the active agent in the *feed additive*. The EURL recommends instead 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.

¹ FAD-2010-0150; ² FAD-2012-0001

KEYWORDS

Enterococcus faecium DSM 10663/NCIMB 10415, E 1707, zootechnical additives, gut flora stabilisers, calves for rearing, chickens for fattening, turkeys for fattening, suckling and weaned piglets, dogs, cats.

1. BACKGROUND

In the current application authorisation is sought under Article 10(2) (re-evaluation of already authorised additives)¹ and 4(1)² for *Enterococcus faecium* DSM 10663/NCIMB 10415 (E 1705), under the category/functional group 4(b), "zootechnical additives/gut flora stabilisers", according to the classification system of Annex I of Regulation (EC) No 1831/2003 [1, 2]. The strain is deposited in the 'Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ)' in Braunschweig, Germany [3].

The *feed additive* will be marketed in several forms at concentration levels of *Enterococcus faecium* DSM 10663/NCIMB 10415 [4,5] listed hereafter:

| Oralin | Form | x10 ¹⁰ CFU/g |
|--------|------------|-------------------------|
| 350 P | powder | 3.5 |
| 350 G | granulated | 3.5 |
| 200 C | coated | 2 |
| 100 S | liquid | 1 |

Specifically, authorisation is sought for the use of the *feed additive* for chickens for fattening¹, calves for rearing¹, piglets (suckling and weaned)¹, turkeys for fattening¹, dogs¹ and cats². The *feed additive* is intended to be used either via *premixtures* or incorporated directly into the *feedingstuffs*. The Applicant proposed the following content of *Enterococcus faecium* DSM 10663/NCIMB 10415 in complete *feedingstuffs*:

- for turkeys for fattening: ranging from 1x10⁷ to 1x10⁹ CFU/kg [4];
- for chickens for fattening: a maximum of 1x10⁹ CFU/kg [4];
- for cats, calves for rearing and piglets (suckling and weaned): ranging from 1x10⁹ to 1x10¹⁰ CFU/kg [4,5];and
- for dogs: ranging from 1x10⁹ to 3.5x10¹⁰ 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

¹ FAD-2010-0150; ² FAD-2012-0001

concerning applications for authorisations 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 *Oralin* 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 characterisation of the strain *Enterococcus faecium* DSM 10663/NCIMB 10415 the Applicant used morphology study and API characterisation [6]. These methods are suitable for the purpose of analysis. However, the EURL recommends instead for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised standard methodology for microbial identification [7].

Qualitative and quantitative composition of impurities in the additive

The Applicant analysed the *feed additive* for microbial contaminants (such as *Escherichia coli*, *Salmonella* spp., yeasts and moulds) using appropriate internationally recognised tests [8]. For undesirable substances (i.e. arsenic, cadmium, mercury, lead, selenium, copper, zinc, chrome and 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 substance in feed additive, premixtures and feedingstuffs

For the enumeration of *Enterococcus faecium* DSM 10663/NCIMB 10415 in the *feed additive, premixtures and feedingstuffs*, the Applicant proposed a ring-trial validated double-layer plate method, using Slanetz and Bartley agar, developed by VDLUFA[9].

The samples are suspended in peptone+Tween sodium phosphate buffer solution (for fat-encapsulated: Tris/Tween peptone solution) and diluted in a phosphate buffer solution. The appropriate dilutions are then transferred into petri dishes and Slanetz and Bartley agar is added in two layers. When the agar is solidified, plates are incubated at 37 °C for 36 hours before colony counting. The method is suitable for enumeration of *Enterococcus faecium* DSM 10663/NCIMB 10415 in different feed matrices.

However, the EURL identified instead the internationally recognised ring-trial validated spread plate method issued by the European Committee for Standardization (CEN) for the enumeration of *Enterococcus* spp (EN 15788) [10]. The samples are suspended in phosphate buffered saline (PBS) and diluted in a peptone salt solution; for fat-encapsulated samples appropriate solution (i.e. polyoxyethylensorbitanmonooleat = Tween) is used. The appropriate dilutions are then spread on Bile Esculin Azide Agar and the agar plates are incubated at 37 °C for 24 hours before colony counting. The performance characteristics of the CEN method reported after logarithmic transformation (CFU) are:

- a *repeatability* standard deviation (s_r) ranging from 0.12 to 0.2 \log_{10} CFU/g;
- a *reproducibility* standard deviation (s_R) ranging from 0.23 to 0.41 \log_{10} CFU/g; and
- a limit of detection (LOD) of 1×10^5 CFU/kg *feedingstuffs* [11].

Therefore, based on the performance characteristics presented, the EURL recommends for official control, the CEN method (EN 15788) for the enumeration of *Enterococcus faecium* DSM 10663/NCIMB 10415 in *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.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control the CEN method (EN 15788) for the enumeration of *Enterococcus faecium* DSM 10663/NCIMB 10415 in the *feed additive*, *premixtures* and *feedingstuffs* and Pulsed Field Gel Electrophoresis (PFGE) for the identification of the strain.

Recommended text for the register entry (analytical method)

Enumeration of *Enterococcus faecium* DSM 10663/NCIMB 10415 in the *feed additive*, *premixtures* and *feedingstuffs*:

- Pour plate method using Bile Esculin Azide Agar (EN 15788)

Identification of *Enterococcus faecium* DSM 10663/NCIMB 10415:

- 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 *Oralin* 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/Ref: SANCO/G1: Forw.Appl.1831/0031-2012
- [2] #Application/Ref: SANCO/G1: Forw.Appl.1831/0004-2012
- [3] *Technical Dossier, Section II, Annex_II_10_DSMZ-Safe deposit
- [4] #Application, Proposal for Register Entry, Annex A
- [5] *Application, Proposal for Register Entry, Annex A
- [6] *#Technical dossier, Section II, 2.2. Characterisation of the active agent
- [7] 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)"
- [8] *#Technical Dossier, Section II.2.1.4. Purity
- [9] VDLUFA Methodenbuch III, 28.2.3. Determination of *Enterococcus faecium*
- [10] EN 15788 'Animal feeding stuffs - Isolation and enumeration of *Enterococcus (E. faecium) spp*'
- [11] ISO 7218:1996 - Microbiology of food and animal feedingstuffs – General rules for microbiological examinations

*Refers to Dossier No: FAD-2010-0150

#Refers to Dossier No: FAD-2012-0001

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

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, as last amended by Regulation (EC) No 885/2009.

8. ACKNOWLEDGEMENTS

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- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes (FR)
- Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim (DE)