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

Dossier related to: FAD-2008-0060
CRL/080031

Name of Additive: *Lactiferm*

Active Agent (s): *Enterococcus faecium M74,*
NCIMB 11181

Rapporteur Laboratory: Community Reference Laboratory for
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EXECUTIVE SUMMARY

In the current application authorisation is sought for the microbial feed additive *Enterococcus faecium M74 NCIMB 11181* under the category 'zootechnical additive', functional group 'gut flora stabilisers' according to Annex I of Regulation (EC) 1831/2003. Specifically, authorisation is sought for the use of *Enterococcus faecium M74 NCIMB 11181* for chickens for fattening. *Enterococcus faecium M74 NCIMB 11181* is provided as a powder or in a coated form containing at least 5×10^{10} CFU/g and also in a water soluble form containing at least 2×10^{11} CFU/g spores of *Enterococcus* as active agent. The feed additive is intended to be mixed into complete *feedingstuffs* at a final concentration of 2.5×10^8 to 1.5×10^{10} CFU/kg of *feedingstuffs*.

For the enumeration of *Enterococcus faecium M74 NCIMB 11181* in the *feed additive*, *premixtures* and *feedingstuffs*, the applicant proposes the CEN method - FprEN 15788 - an internationally recognised spread plate method. This method was ring-trial validated using *feedingstuffs* samples containing *Enterococcus faecium* containing *enterococci* at two different concentrations that cover the target levels of this application. The performance characteristics of the CEN method for the enumeration are, a standard deviations for repeatability (s_r) and reproducibility (s_R) of around $0.12 - 0.20 \log_{10}$ and $0.23 - 0.41 \log_{10}$ (calculated from the base 10 logarithms of the measured CFU/g) in *feedingstuffs*, respectively. The limits of quantification (LOQ) of this method are around 10^7 CFU/kg of *feedingstuffs*.

The applicant used the above mentioned spread plate method to analyse various matrices containing *Enterococcus faecium M74 NCIMB 11181* and reported results at concentrations ranging:

- from 6.8×10^{10} to 1.1×10^{11} (for powder form and coated form) and 2.9×10^{11} to 3.4×10^{11} (in water soluble) CFU/g for *feed additive*;
- from 1.8×10^{12} to 3.9×10^{12} CFU/kg for *premixtures* .
- from 1.7×10^8 to 6.7×10^{11} (for powder and coated form), and 3.9×10^{11} to 8.3×10^{12} (in water soluble), CFU/kg for *feedingstuffs*.

The CRL recommends the above mentioned draft CEN method for official control of the active agent in *feed additive*, *premixtures* and *feedingstuffs*.

The molecular method, 1-D Protein Gel Electrophoresis (SDS-PAGE) was used by the applicant for identification of the active agent. The CRL recommends for official control pulsed field gel electrophoresis (PFGE), a generally recognised standard methodology for microbial identification, is recommended. The CEN Technical Committee 327 is currently developing a European Standard for this methodology. Further testing or validation is not considered necessary.

KEYWORDS

Enterococcus faecium M74 NCIMB 11181, zootechnical, gut flora stabiliser, chicken for fattening.

1. BACKGROUND

Enterococcus faecium M74 NCIMB 11181 is a feed additive for which authorisation is sought under the category of 'zootechnical additives' functional group 'gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003 [1]. *Enterococcus faecium M74 NCIMB 11181* is provided as a powder or in a coated form containing at least 5×10^{10} CFU/g and also in a water soluble form containing at least 2×10^{11} CFU/g of *Enterococcus* as active agent [2]. The strain of *Enterococcus faecium M74 NCIMB 11181* is deposited at the NCIMB (National Collection of Industrial and Marine Bacteria), Aberdeen, Scotland, where is registered as NCIMB 11181 [3]. The intended use of the current application is for chickens for fattening. The proposed recommend dosage is of 2.5×10^8 to 1.5×10^{10} CFU/ kg of complete feedstuffs [2].

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 Community Reference Laboratory concerning applications for authorizations of *feed additives*, the CRL is requested to submit a full evaluation report to the European Food Safety Authority (EFSA) for each application. For this particular dossier, the methods of analysis submitted in connection with the *Enterococcus faecium M74 NCIMB 11181* dossier 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 the identification of *Enterococcus faecium* M74 NCIMB 11181 molecular methods such as SDS-PAGE (1 D-protein gel electrophoresis) and cluster analysis were used by the applicant [4].

For official controls pulsed field gel electrophoresis (PFGE), a generally recognised standard methodology for microbial identification, for official controls pulsed field gel electrophoresis (PFGE), a generally recognised standard methodology for microbial identification, is recommended. The CEN Technical Committee 327 is currently developing a European Standard for this methodology.

Qualitative and quantitative composition of any impurities in the additive

The applicant analysed the *feed additive* for microbial contaminants such as coliform bacteria, *Escherichia coli*, *Salmonella*, yeast and moulds using appropriate EN methods [5]. For undesirable substances (i.e. lead, arsenic, mercury, cadmium, aflatoxins, ochratoxin) internationally recognised standard methods are available at the respective Community Reference Laboratories [6].

Description of the analytical methods for the determination of active agent(s) in feed additive, premixtures and feedingstuffs

For the enumeration of *Enterococcus faecium* M74 NCIMB 11181 in the *feed additive*, *premixtures* and *feedingstuffs*, the applicant proposes the CEN method - FprEN 15788 - an internationally recognised spread plate method. This method was ring-trial validated using *feedingstuffs* samples containing *Enterococcus faecium*. The performance characteristics of the CEN method for the enumeration are a standard deviations for repeatability (s_r) and reproducibility (s_R) of around 0.12 – 0.20 \log_{10} and 0.23 – 0.41 \log_{10} (calculated from the base 10 logarithms of the measured CFU/g) in *feedingstuffs*, respectively. The limit of quantification (LOQ) of this method is around 10^7 CFU/kg of *feedingstuffs* [7].

For the enumeration of *Enterococcus faecium* in *feedingstuffs* an initial suspension of the sample was prepared in a diluent with suitable buffer capacity, the appropriated dilutions were spread on Bile Esculin Azide Agar. The agar plates are incubated at 37 °C for 24 hours.

The applicant used the above mentioned spread plate method to analyse various matrices containing *Enterococcus faecium* M74 NCIMB 11181 at concentrations ranging:

- from 6.8×10^{10} to 1.1×10^{11} (in powder and coated form) and 2.9×10^{11} to 3.4×10^{11} (in water soluble form) CFU/g of *feed additive*;
- from 1.8×10^{12} to 3.9×10^{12} (in powder and coated form) CFU/kg for *premixtures* ; and
- from 1.7×10^8 to 6.7×10^{11} (in powder and coated form), and 3.9×10^{11} to 8.3×10^{12} (in water soluble form), CFU/kg of *feedingstuffs*.

The CRL recommends the draft CEN method for official control of the active agent in *feed additive*, *premixtures* and *feedingstuffs*.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the CRL recommends the draft CEN method - FprEN 15788 - for the enumeration of the active agent *Enterococcus faecium* M74 NCIMB 11181 in the *feed additive*, *premixtures* and *feedingstuffs*.

For the analysis of the identity of the bacterial strain, *Enterococcus faecium* M74 NCIMB 11181 the CRL recommends pulsed-field gel electrophoresis (PFGE) for official control.

Further testing or validation is not considered necessary.

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

- Enumeration: (FprEN 15788) Spread plate method using Bile Esculin Azide agar.
- Identification: Pulsed Field Gel Electrophoresis (PFGE)

5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

In accordance with the requirements of Regulation (EC) No 1831/2003, samples of the additive *Enterococcus faecium* M74 NCIMB 11181 for chickens for fattening have been sent to the Community Reference Laboratory for Feed Additives Authorisation. The dossier has been made available to the CRL by EFSA.

6. REFERENCES

- [1] * Application/Ref:SANCO/D/2:Forw.Appl.1831/039-2008
- [2] * Proposal of Register entry EFSA Annex A
- [3] * Technical dossier, section II/ 2.2. Characterisation of the active substance(s)/Agent(s)
- [4] * Annex II.2.1 Identification
- [5] * Technical dossier, section II/ 2.6 Method of analysis and reference samples
- [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 Community reference laboratories, Official Journal of the European Union L 136
- [7] Draft CEN method (FprEN 15788:2009) Animal feeding stuffs - Isolation and enumeration of Enterococcus (*E. faecium*) spp. (version April 2009)

*Refers to Dossier no: FAD-2008-0060

7. RAPPORTEUR LABORATORY

The Rapporteur Laboratory for this evaluation was Community 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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