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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-2009-0024
CRL/090014

Active Agent(s): *Pediococcus pentosaceus* DSM 16244

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EXECUTIVE SUMMARY

In the current application re-evaluation of the authorisation is sought for the microbial feed additive *Pediococcus pentosaceus* DSM 16244 under the category 'technological additives', functional group 'silage additive' according to Annex I of Regulation (EC) No 1831/2003. The *feed additive* is a powder intended to be mixed at a minimum dose of 1×10^5 CFU/g of fresh matter ensilage for all species.

For the determination of *Pediococcus pentosaceus* DSM 16244 in the *feed additive per se* the applicant submitted a single laboratory validated method, adapted from the ISO method 15214 ("*Microbiology of food and animal feeding stuffs - horizontal method for the enumeration of mesophilic lactic acid bacteria*") and reported satisfactory performance characteristics. Furthermore, the applicant applied the method described by Leuschner *et al* (2003) (presently available as CEN method EN 15787:2009 "*Animal feeding stuffs - isolation and enumeration of Lactobacillus spp*"), and reported comparable results and performance characteristics, thus confirming and extending the applicability of the CEN method for the enumeration in the *feed additive per se*. However, the same experimental protocol - spread plate technique on MRSA agar - is used in the CEN method EN 15786:2009 specifically dedicated to the "*enumeration of Pediococcus spp*". The performance characteristics of this ring trial validated method - reported after \log_{10} transformation - are:

- a repeatability standard deviation (s_r) ranging from 0.01 to 0.17 \log_{10} CFU/g, and
- a reproducibility standard deviation (s_R) ranging from 0.10 to 0.26 \log_{10} CFU/g.

Based on these acceptable performance characteristics, the CRL recommends for official control the dedicated CEN method EN 15786:2009 for the determination of *Pediococcus pentosaceus* DSM 16244 in the *feed additive per se*.

As mentioned above two CEN standard methods are available for the enumeration of pediococci in feedingstuffs: (a) EN 15787:2009 for "lactobacillus spp", and (b) EN 15786:2009 for "pediococcus spp". However neither experimental data nor standard methods are available for the determination of *Pediococcus pentosaceus* DSM 16244 in the silage matrix.

Molecular methods were used by the applicant for identification of the active agent in the *feed additive* and in *silage*. The CRL recommends for official control pulsed field gel electrophoresis (PFGE), a generally recognised standard methodology for microbial

identification. The CEN Technical Committee 327 is currently developing a European Standard for this methodology.

Further testing or validation is not considered necessary.

KEYWORDS

Pediococcus pentosaceus DSM 16244, technological additives, silage additive.

1. BACKGROUND

Pediococcus pentosaceus DSM 16244 is a feed additive for which a re-evaluation of the authorisation (Article 10(2) or 10(7)) is sought under the category (1k) 'technological additives', functional group 'silage additive' according to Annex I of Regulation (EC) No 1831/2003 [1]. The strain originally isolated from natural silage is not genetically modified, and is deposited at culture collection, on the 'Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH' (DSMZ, Braunschweig, Germany) as DSM 16244 [2]. The *feed additive* is a powder intended to be mixed at a minimum dose of 1×10^5 CFU/g of fresh matter ensilage for all species [3].

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 dossier, the methods of analysis submitted in connection with the *Pediococcus pentosaceus* DSM 16244 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

The applicant used Pulsed Field Gel Electrophoresis (PFGE) for identification and characterization of the strain *Pediococcus pentosaceus* DSM 16244 in *feed additive*. This method is suitable for the purpose of analysis [4].

The CRL recommends for official control PFGE, a generally recognised standard methodology, for identification and characterization of the strain *Pediococcus pentosaceus* DSM 16244 in *feed additive* and in *silage*. The CEN Technical Committee 327 is currently developing a European Standard for the PFGE methodology.

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* and moulds) by using appropriate EN ISO tests [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 in silage additive

The applicant submitted a single laboratory validated method, adapted from the ISO method 15214 [7] for the determination of *Pediococcus pentosaceus* DSM 16244 in the *feed additive per se*, using a spread plate technique with MRS (de Man, Rogosa, Sharp) agar. The feed additive powdery sample was suspended, diluted, transferred to the MRS agar plate and incubated for 72 hours at 37°C before counting. The following performance characteristics for the single laboratory validated method were reported by the applicant [8]:

- a repeatability standard deviation (s_r) of 0.02 log₁₀ CFU/g
- a reproducibility intermediate precision of 0.03 log₁₀ CFU/g, and

- a limit of quantification (LOQ) of 10 CFU/g of feed additive.

Furthermore, the applicant applied the method developed by Leuschner *et al.* [9] for the determination of *Pediococcus pentosaceus* DSM 16244 in the *feed additive per se*. This method is presently available as CEN method EN 15787:2009 [10]. Comparable results and performance characteristics were reported [11], thus confirming and extending the applicability of the CEN method EN 15787:2009 for the enumeration to the *feed additive* matrix. However, the same experimental protocol (spread plate technique on MRSA agar) is used in the CEN method EN 15786:2009 [12] specifically dedicated to the "enumeration of *Pediococcus* spp". The performance characteristics of this ring trial validated method - reported after \log_{10} transformation - are:

- a repeatability standard deviation (s_r) ranging from 0.01 to 0.17 \log_{10} CFU/g, and
- a reproducibility standard deviation (s_R) ranging from 0.10 to 0.26 \log_{10} CFU/g.

Based on these acceptable performance characteristics, the CRL recommends for official control the dedicated CEN method EN 15786:2009 for the determination of *Pediococcus pentosaceus* DSM 16244 in the *feed additive per se*.

As mentioned earlier two CEN standard methods are available for the enumeration of *Pediococcus* in *feedingstuffs*: (a) EN 15787:2009 for "Lactobacillus spp", and (b) EN 15786:2009 for "*Pediococcus* spp". However neither experimental data nor standard methods are available for the determination of *Pediococcus pentosaceus* DSM 16244 in the silage matrix.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the CRL recommends the CEN method (EN 15786:2009) for the enumeration of the active agent *Pediococcus pentosaceus* DSM 16244 in the *feed additive*. For the analysis of the identity of the bacterial strain *Pediococcus pentosaceus* DSM 16244 in *feed additive* and in *silage*, the CRL recommends Pulsed Field Gel Electrophoresis (PFGE) for official control. The CEN Technical Committee 327 is currently developing a European Standard for this methodology.

Further testing or validation is not considered necessary.

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

- Enumeration in *feed additive*:

Spread plate method using MRS agar at 37°C (EN15786:2009)

- Identification in *feed additive* and in *silage*:

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 *Pediococcus pentosaceus* DSM 16244 have been sent to the Community Reference Laboratory for Feed Additives Authorization. The dossier has been made available to the CRL by EFSA.

6. REFERENCES

- [1] * Application, Ref: SANCO/D/2: Forward Application. 1831/019-2009
- [2] * Technical dossier section II.2- "Characterization of the active substance(s) /agent(s)"
- [3] * Application, Proposal of Registry Entry Annex A
- [4] * Technical dossier Annex II.2.2.
- [5] * Technical dossier Annex II.1-3,1-4
- [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] ISO 15214:1998 - Microbiology of food and animal feeding stuffs -horizontal method for the enumeration of mesophilic lactic acid bacteria – Colony count techniques at 30 degrees C
- [8] * Technical dossier - Annex-II.6.1 "Methods of analysis and reference samples"
- [9] * Technical dossier - Annex-II.6.3 - Leuschner *et al.* Food Microbio. 20 (2003) 57
- [10] EN 15787:2009 - Animal feeding stuffs. Isolation and enumeration of *Lactobacillus* spp.
- [11] * Technical dossier - Annex II.6.2 – Report
- [12] EN 15786:2009 - "Animal feeding stuffs- isolation and enumeration of *Pediococcus* spp"

*Refers to Dossier no: FAD-2009-0024

7. RAPPORTEUR LABORATORY

The Rapporteur Laboratory for this evaluation was the National Reference Laboratory for Feed Additive (NRL), CreAA, Istituto Zooprofilattico, Torino, Italy. 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. ACKNOWLEDGEMENT

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- Thüringer Landesanstalt für Landwirtschaft , Abteilung Untersuchungswesen. Jena (DE)