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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**

**Ten "*micro-organisms used as silage agents*"
(i.e. 3 lactobacilli and 7 pediococci)**

FAD	CRL
FAD-2010-0127	CRL/100126
FAD-2010-0252	CRL/100342
FAD-2010-0259	CRL/100335
FAD-2010-0280	CRL/100282

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Date: **07/11/2011**

EXECUTIVE SUMMARY

This report is on the evaluation of feed additives "*micro-organisms used as silage agents*", which is related to the application of ten *micro-organisms* for which authorisation is sought under Article 10(7). Authorisation is sought for all the above mentioned *micro-organisms* under category/functional group 1(k), "technological additives/silage additives", according to Annex I of Regulation (EC) No 1831/2003. The list of *micro-organisms* of interest and the minimum activities in the *feed additives* and in *silage*, as sought in the authorisation, are presented in Table 1^(*). The intended use of the current applications is for all animal species.

For identification and characterisation of all ten *micro-organisms* of concern (i.e. *lactobacilli* and *pediococci*) the EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised standard methodology for microbial identification.

The EURL recommends for enumeration in the *feed additives* the following ring trial validated methods:

- Spread plate method using MRS agar (EN 15787) for *Lactobacilli*; and
- Spread plate method using MRS agar (EN 15786) for *Pediococci*.

None of the Applicants provided experimental data for the determination of *micro-organisms* in *silage*. Furthermore, the unambiguous determination of the content of *micro-organisms* added to *silage* is not achievable by analysis. Therefore the EURL cannot evaluate nor recommend any method for official control to determine any of the ten *micro-organisms* of concern 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.

(*)Full list provided in EURL evaluation report, available from the EURL website.

KEYWORDS

ten *micro-organisms* (listed in Table 1), technological additives, silage additives, all animal species.

1. BACKGROUND

In the current application authorisation is sought under Article 10(7) (re-evaluation of already authorised additives) for ten "*micro-organisms used as silage agents*", under the category/functional group 1(k), "technological additives/silage additives", according to Annex I of Regulation (EC) No 1831/2003 [1].

The list of *micro-organisms* of interest is presented in Table 1 which includes:

- the species as currently specified in the Community register;
- the species as proposed by the Applicant [2];
- the name of the Institute where the original strain is deposited [3]; and
- the minimum activities in the *feed additive* and in *silage* as sought in the authorisation [2].

Specifically, authorisation is sought for the *feed additive* to be placed on the market in the form of powder [4]. The intended use of the current applications is for all animal species.

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 ten *micro-organisms* listed in Table 1 and their suitability to be used for official controls in the frame of the authorisation were evaluated.

Table 1: List of *micro-organisms* with the corresponding minimum activities in the *feed additive* (a) and in *silage* (b) as proposed in Annex A [2]

FAD	micro-organisms	as found in COM Register	as proposed by Applicant	deposited @	(a) CFU/g	(b) CFU/kg
FAD-2010-0127	Pediococcus pentosaceus	NCIMB 30171	5M-1P – NCIMB 30171 - DSM 23376	DSMZ	NA	1.0E+08
	Pediococcus acidilactici	CNCM I-3237 / ATCC 8042	ATCC 8042 - CNCM I-3237	CNCM	2.0E+10	1.4E+07
	Pediococcus acidilactici	(*) CNCM MA 18/5M or DSM 11673	CNCM MA 18/5M - DSM 11673	CNCM DSMZ	3.0E+09	3.0E+07
	Pediococcus pentosaceus	NCIMB 12455	NCIMB 12455	NCIMB	3.0E+09	3.0E+07
	Pediococcus pentosaceus	MBS-PP-01	MBS-PP-01 – NCIMB 30237	NCIMB	NA	8.0E+07
	Pediococcus pentosaceus	NCIMB 30168	NCIMB 30168	NCIMB	5.0E+10	1.0E+08
FAD-2010-0252	Pediococcus	pentosaceus NCIMB 30089	acidilactici NCIMB 30089	NCIMB	1.0E+11	5.0E+05
FAD-2010-0259	Lactobacillus plantarum	NCIMB 40027	NCIMB 40027	NCIMB	1.0E+11	1.0E+05
FAD-2010-0280	Lactobacillus plantarum	Aber F1 NCIMB 41028	Aber F1 NCIMB 41028	NCIMB	7.0E+10	1.0E+09
	Lactobacillus plantarum	L54 NCIMB 30148	L54 NCIMB 30148	NCIMB	7.0E+10	1.0E+09

Major differences in nomenclature highlighted

NA: Not Available

*According to the Applicant, two strains of *Pediococcus acidilactici* are synonymous they are currently listed as two separate entries in Community Register of Feed Additives

Culture Collections**CNCM:** Collection Nationale De Cultures De Microorganismes (FR);**DSMZ:** Deutsche Sammlung von Mikroorganismen und Zellkulturen (DE);**NCIMB:** The National Collection of Industrial, food and Marine Bacteria (UK).

3. EVALUATION

Identification/Characterisation of the feed additive

Qualitative and quantitative composition of the additive

For identification and characterisation of all 10 *micro-organisms* of concern listed in Table 1 (cf. *Lactobacilli* and *Pediococci*) the EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised standard methodology for microbial identification [5].

Qualitative and quantitative composition of any impurities in the additive

The Applicants analysed the *feed additive* for microbial contaminants (such as Enterobacteria, *Escherichia coli*, *Salmonella* spp. and yeasts) by using appropriate EN ISO tests. 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

a. Lactobacilli

For enumeration of the 3 *Lactobacilli* in the *feed additive*, the Applicants proposed the ring trial validated spread plate method EN 15787 [6]. The sample is suspended and diluted in a phosphate buffered saline (PBS); the appropriate dilutions are then spread on MRS agar plates. The agar plates are incubated at 37 °C for 48 to 72 hours. The performance characteristics of the CEN method reported after logarithmic transformation are [6]:

- a standard deviation for repeatability (S_r) of 0.24 \log_{10} CFU/g;
- a standard deviation for reproducibility (S_R) ranging from 0.29 to 0.38 \log_{10} CFU/g; and
- a limit of detection (LOD) of 10^5 CFU/kg *feedingstuffs* [7].

Based on the performance characteristics presented the EURL recommends for official control the CEN method (EN 15787) for the determination of the 3 *Lactobacilli* of interest in the *feed additive per se*.

b. Pediococci

For enumeration of the 7 *pediococci* of interest in the *feed additive*, the Applicants proposed the ring-trial validated spread plate method EN 15786 [8]. The sample is suspended and diluted in PBS; the appropriated dilutions are then spread on MRS agar plates. The agar plates are incubated at 37 °C for 48 hours. The performance characteristics of the CEN method reported after logarithmic transformation are [8]:

- S_r ranging from 0.01 to 0.17 \log_{10} CFU/g;
- S_R ranging from 0.10 to 0.26 \log_{10} CFU/g; and
- LOD = 10^5 CFU/kg [7].

Based on the performance characteristics presented the EURL recommends for official control the CEN method (EN 15786) for the determination of the 7 *pediococci* of interest in the *feed additive per se*.

None of the Applicants provided experimental data for the determination of *micro-organisms* in *silage*. Furthermore, the unambiguous determination of the content of *micro-organisms* added to *silage* is not achievable by analysis. Therefore the EURL cannot evaluate nor recommend any method for official control to determine any of the ten *micro-organisms* of concern 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 Pulsed Field Gel Electrophoresis (PFGE) for the identification of the 10 *micro-organisms* of concern.

The EURL recommends for enumeration in the *feed additives* the following methods:

- Spread plate method using MRS agar (EN 15787) for *Lactobacilli*; and
- Spread plate method using MRS agar (EN 15786) for *Pediococci*.

None of the Applicants provided experimental data for the determination of *micro-organisms* in *silage*. Furthermore, the unambiguous determination of the content of *micro-organisms* added to *silage* is not achievable by analysis. Therefore the EURL cannot evaluate nor recommend any method for official control to determine any of the ten five *micro-organisms* of concern in *silage*.

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

Enumeration in the *feed additive* of

- 3 *Lactobacilli*:
EN 15787 - Spread plate method using MRS agar
- 7 *Pediococci*:
EN 15786 - Spread plate method using MRS agar

Identification of ten *micro-organisms* of concern:

- 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 ten *micro-organisms* of concern, 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/G1:

SANCO Ref.	Dossier
Forw.Appl 1831/0023-2011	FAD-2010-0127
Forw.Appl 1831/0024-2011	FAD-2010-0252
Forw.Appl 1831/0026-2011	FAD-2010-0259
Forw.Appl 1831/0027-2011	FAD-2010-0280

- [2] *Application, Annex A, Proposal for register entry
 - [3] *Technical Dossier, Section II.2.2. Characterisation of the active substance(s)/agent(s)
 - [4] *Technical Dossier, Section II.2.1. Identity of the additive
 - [5] 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)
 - [6] EN 15787:2009 - Animal feeding stuffs. Isolation and enumeration of *Lactobacillus* spp
 - [7] ISO 7218:1996 - Microbiology of food and animal feedingstuffs – General rules for microbiological examinations
 - [8] EN 15786:2009 - Animal feeding stuffs. Isolation and enumeration of *Pediococcus* spp
- *Refers to all four FAD Dossiers, listed under [1]

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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- Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim (DE)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Państwowy Instytut Weterynaryjny, Puławy (PL)
- Sächsische Landesanstalt für Landwirtschaft, Fachbereich 8 — Landwirtschaftliches Untersuchungswesen, Leipzig (DE)