



**EUROPEAN COMMISSION**  
JOINT RESEARCH CENTRE

Directorate F - Health, Consumers & Reference Materials (Geel/Ispra)  
**European Union Reference Laboratory for Feed Additives**

JRC F.5/CvH/MGH/AS/Ares

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

**Propionibacterium freudenreichii ssp. shermanii (ATCC PTA-6752)**  
*(FAD-2017-0040; CRL/170006)*





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in connection with the Application for Authorisation of a  
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Dossier related to: **FAD-2017-0040 - CRL/170006**

Name of Product: ***Propionibacterium freudenreichii ssp.  
shermanii* (ATCC PTA-6752)**

Active Agent (s): ***Propionibacterium freudenreichii ssp.  
shermanii* (ATCC PTA-6752)**

Rapporteur Laboratory: **Centre wallon de Recherches  
agronomiques (CRA-W), Gembloux,  
Belgium**

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Date: **17/12/2018**

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Date: **17/12/2018**

## EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4(1) for *Propionibacterium freudenreichii* ssp. *shermanii* (ATCC PTA-6752) (PF24) under the category / functional group 1(j) 'technological additives' / 'acidity regulators', according to Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for the use of the *feed additive* for all animal species.

According to the Applicant, the *feed additive* (PF24) contains as active substance viable cells of the non-genetically modified strain *Propionibacterium freudenreichii* ssp. *shermanii* (ATCC PTA-6752). The *feed additive* is to be marketed as a powder containing a minimum *Propionibacterium freudenreichii* ssp. *shermanii* (ATCC PTA-6752) content of  $4 \times 10^{11}$  Colony Forming Unit (CFU)/g. The *feed additive* is intended to be used directly in *feedingstuffs* at minimum doses of  $1 \times 10^9$  CFU/l for liquid *feedingstuffs*, and of  $2 \times 10^9$  CFU/kg for dry *feedingstuffs*.

For the identification of *Propionibacterium freudenreichii* ssp. *shermanii* (ATCC PTA-6752), the EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised methodology for the genetic identification of bacterial strains.

For the enumeration of *Propionibacterium freudenreichii* ssp. *shermanii* (ATCC PTA-6752) in *feed additive* and *feedingstuffs*, the Applicant submitted an in-house colony-count method, single-laboratory validated and further verified by an external laboratory. Based on the performance characteristics reported, the EURL recommends this method for official control.

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

*Propionibacterium freudenreichii* ssp. *shermanii* (ATCC PTA-6752), technological additives, acidity regulators, all animal species

## 1. BACKGROUND

In the current application authorisation is sought under Article 4(1) for *Propionibacterium freudenreichii* ssp. *shermanii* (ATCC PTA-6752) (PF24) under the category / functional group 1(j) 'technological additives' / 'acidity regulators', according to Annex I of Regulation (EC) No 1831/2003 [1]. Authorisation is sought for the use of the *feed additive* for all animal species [1, 2].

According to the Applicant, the *feed additive* 'PF24' contains as *active substance* viable cells of the non-genetically modified strain *Propionibacterium freudenreichii ssp. shermanii* (ATCC PTA-6752) [3]. The strain is deposited at the American Type Culture Collection (ATCC, Manassas, VA, USA) under the deposit number PTA-6752 [4].

The *feed additive* is to be marketed as a powder containing a minimum *Propionibacterium freudenreichii ssp. shermanii* (ATCC PTA-6752) content of  $4 \times 10^{11}$  Colony Forming Unit (CFU)/g [5].

The *feed additive* is intended to be used directly in *feedingstuffs* at minimum doses of  $1 \times 10^9$  CFU/l for liquid *feedingstuffs*, and of  $2 \times 10^9$  CFU/kg for dry *feedingstuffs* [6].

Note: The EURL previously evaluated the analytical methods for the determination of *Propionibacterium ssp.* in the frame of dossier FAD-2010-0255 [7].

## 2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761, 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 the tasks of the European Union Reference Laboratory concerning applications for authorisations of feed additives, the EURL is requested to submit a full evaluation report to the European Food Safety Authority for each application or group of applications. For this particular dossier, the methods of analysis submitted in connection with *Propionibacterium freudenreichii ssp. shermanii* (ATCC PTA-6752) and their suitability to be used for official controls in the frame of the authorisation were evaluated.

## 3. EVALUATION

***Description of the analytical methods for the determination of the active substance in the feed additive, premixtures, feedingstuffs and when appropriate water (section 2.6.1 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)***

For the enumeration of *Propionibacterium freudenreichii ssp. shermanii* (ATCC PTA-6752) in the *feed additive* and in *feedingstuffs*, the Applicant submitted an in-house colony-count method, single-laboratory validated and further verified by an external laboratory for the *feed additive* (PF24) and dry feedingstuffs [8, 9, 10]. In addition, the Applicant demonstrated the suitability of the proposed method for liquid feedingstuffs in the frame of stability and homogeneity studies in liquid feeds [11].

The sample is suspended and diluted in a peptone-salt solution. Appropriate dilutions are spread on lactate agar plates and incubated anaerobically at 30 °C for 7 days. Brown coloured colonies are then counted on plates containing at least 30 colonies.

**Table 1:** Performance characteristics of analytical methods for the determination of (*PF24*) in *feed additive* (FA) and *feedingstuffs* (FS)

Matrix	Bacterial Counts (CFU/g)		Sr (log <sub>10</sub> CFU/g)		S <sub>ip</sub> (log <sub>10</sub> CFU/g)	
	Val/	Ver/	Val	Ver	Val	Ver
FA	6x10 <sup>11</sup>	7x10 <sup>11</sup>	0.04	0.06-0.08	0.05	0.07
FS	5x10 <sup>8</sup>	4x10 <sup>8</sup>	0.06	0.04-0.09	0.07	0.07

S<sub>r</sub>: standard deviation for repeatability; S<sub>ip</sub> standard deviation for intermediate precision;  
Val: Validation; Ver: verification

The main performance characteristics presented by the Applicant in the frame of the validation and verification [9] studies are summarised in Table 1. Additionally the Applicant reported a limit of quantification (LOQ) of 3x10<sup>3</sup> CFU/g.

Based on the performance characteristics provided by the Applicant, the EURL recommends for official control the single-laboratory validated and further verified colony-count method for the enumeration of *Propionibacterium freudenreichii ssp. shermanii* (ATCC PTA-6752) in the *feed additive* and in *feedingstuffs*.

Note: The Applicant demonstrated that the analytical method CEN 15787, previously evaluated by the EURL for the enumeration of *Propionibacterium* spp., is not suitable for the specific strain subject of this application.

***Methods of analysis for the determination of the residues of the additive in food (section 2.6.2 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)***

The evaluation of corresponding methods of analysis is not relevant for the present application.

***Identification/Characterisation of the feed additive (section 2.6.3 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)***

For the identification of *Propionibacterium freudenreichii ssp. shermanii* (ATCC PTA-6752), the Applicant applied 16S rRNA gene sequence analysis [3].

The EURL recommends instead for official control the Pulsed-Field Gel Electrophoresis (PFGE), a generally recognised methodology for genetic identification of bacterial strains [13].

This methodology for microbial identification is currently being evaluated by the CEN Technical Committee 327 to become an European Standard.

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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

#### 4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE) for the identification of *Propionibacterium freudenreichii ssp. shermanii* (ATCC PTA-6752) and the single-laboratory validated and further verified colony-count method for enumeration of this strain in the *feed additive* and *feedingstuffs*.

##### *Recommended text for the register entry (analytical method)*

- Identification: Pulsed Field Gel Electrophoresis (PFGE)
- Enumeration in *feed additive* and *feedingstuffs*: single-laboratory validated and further verified spread plate method on lactate agar incubated anaerobically at 30 °C for 7 days.

#### 5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Propionibacterium freudenreichii ssp. shermanii* (ATCC PTA-6752) 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, Reference SANTE E5: F.A. 1831/0031-2017
- [2] \*Application, Proposal for Register Entry, Annex A
- [3] \*Technical dossier, Section II: 2.2 Characterisation of the Active Substance
- [4] \*Technical dossier, Section II: Annex II.2.1.2
- [5] \*Technical dossier, Section II: 2.1.3 Qualitative and quantitative composition
- [6] \*Technical dossier, Section II: 2.5 Conditions of use of the additive
- [7] EURL Evaluation Reports:  
<https://circabc.europa.eu/sd/a/06d4f748-155c-47d1-94d0-d571be7c3187/FinRep-FAD-2010-0255-Propionibacterium.pdf>
- [8] \*Supplementary information: Annex II.6.1.updated and Annex II.6.1.2
- [9] \*Supplementary information: Annex II.6.1.1 and Annex II.6.1.3
- [10] \*Supplementary information: SOP-06773\_v1
- [11] \*Supplementary information: Annex II.4.1.3 and Annex II.4.2
- [12] 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) and Report 20873/3 EN (2002) ISBN 92-894-6252-3 (Vol. III)

\*Refers to Dossier no: FAD-2017-0040

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## 7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation is the Centre wallon de Recherches agronomiques (CRA-W), Gembloux, 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 (EU) 2015/1761.

## 8. ACKNOWLEDGEMENTS

The following National Reference Laboratories contributed to this report:

- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Laboratoire de Rennes (SCL L35), Service Commun des Laboratoires DGCCRF et DGDDI, Rennes (FR)
- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, Pesca, Alimentació i Medi Natural. Generalitat de Catalunya, Cabrils (ES)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)