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# Safety and efficacy of TechnoSpore® (*Bacillus coagulans* DSM 32016) for piglets, other growing Suidae, chickens for fattening, other poultry for fattening and ornamental birds

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Maryline Kouba, Mojca Kos Durjava, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Noël Dierick, Giovanna Martelli, Pier Sandro Cocconcelli, Boet Glandorf, Lieve Herman, Miguel Prieto Maradona, Maria Saarela, Montserrat Anguita and Rosella Brozzi

## Abstract

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of TechnoSpore® (*Bacillus coagulans* DSM 32016), when used as a zootechnical additive for piglets (suckling and weaned), other growing Suidae, chickens for fattening, other poultry for fattening and ornamental birds. The bacterial species present in the additive is considered suitable for the qualified presumption of safety approach to safety assessment. The identity of the active agent was established and the lack of toxigenic potential confirmed. *B. coagulans* DSM 32016 did not show resistance to antibiotics of human and veterinary importance, and therefore, was presumed safe for the target species, consumers of products derived from animals Fed the additive and the environment. Since the other components of the additive did not give rise to concerns, TechnoSpore® was also considered safe for the target species, consumer and the environment. The additive is not a skin/eye irritant or a skin sensitiser but should be considered a respiratory sensitiser. TechnoSpore® showed the potential to be efficacious as a zootechnical additive in weaned piglets and chickens for fattening at $1 \times 10^9$ CFU/kg complete feed. This conclusion was extended to suckling piglets and extrapolated to other growing Suidae at the same physiological stage and to other birds for fattening and ornamental birds at the same use level. *B. coagulans* DSM 32016 included in Technospore® is compatible with halofuginone and diclazuril. The Panel could not conclude on the compatibility of the additive with monensin sodium, decoquinate, robenidine hydrochloride, lasalocid sodium, narasin, salinomycin sodium, maduramicin ammonium, nicarbazin and narasin/nicarbazin.



**Keywords:** zootechnical additive, gut flora stabiliser, TechnoSpore®, *Bacillus coagulans*, safety, efficacy, QPS

**Requestor:** European Commission

**Question number:** EFSA-Q-2019-00313

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# 1. Introduction

## 1.1. Background and Terms of Reference as provided by the requestor

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Biochem Zusatzstoffe Handels- und Produktionsges. mbH[2] for authorisation of the product TechnoSpore® (*Bacillus coagulans* DSM 32016), when used as a feed additive for piglets (suckling and weaned), other growing *Suidae*, chickens for fattening, other poultry for fattening and ornamental birds (category: zootechnical additives; functional group: gut flora stabilisers).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). The particulars and documents in support of the application were considered valid by EFSA as of 31 July 2019.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the product TechnoSpore® (*Bacillus coagulans* DSM 32016), when used under the proposed conditions of use (see 3.1.4).

## 1.2. Additional information

TechnoSpore® is a preparation of *Bacillus coagulans* DSM 32016. It has not been previously authorised as a feed additive in the EU.

# 2. Data and methodologies

## 2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[3] in support of the authorisation request for the use of TechnoSpore® (*Bacillus coagulans* DSM 32016) as a feed additive.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active agent in animal feed. The Executive Summary of the EURL report can be found in Annex A.[4]

## 2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of TechnoSpore® (*Bacillus coagulans* DSM 32016) is in line with the principles laid down in Regulation (EC) No 429/2008[5] and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018a) and Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018b).

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] Biochem Zusatzstoffe Handels- und Produktionsges. mbH, Küstermeyerstr. 16, 49393, Lohne, Germany.
[3] FEED dossier reference: FAD-2019-0024.
[4] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2019-0024-technospore.pdf
[5] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

# 3. Assessment

The additive is a preparation containing viable spores of a strain of *Bacillus coagulans* to be used in feed for suckling and weaned piglets, other growing Suidae, chickens for fattening, other poultry for fattening and ornamental birds as a zootechnical additive (functional group: gut flora stabilisers).

## 3.1. Characterisation

### 3.1.1. Characterisation of the active agent

The active agent was isolated from canned tomatoes and is deposited with the Deutsche Sammlung für Mikroorganismen und Zellkulturen under the accession number DSM 32016.[6]

The full genome of *B. coagulans* DSM 32016 was sequenced. The taxonomic identification of the strain was achieved [redacted][7] No cytotoxic effects were detected in a cytotoxicity test performed [redacted] in accordance with the FEEDAP guidance (EFSA FEEDAP Panel, 2018a).[8]

The susceptibility of the strain to the antibiotics recommended by the FEEDAP Panel was tested by broth microdilution following the method of the Clinical and Laboratory Standards Institute (CLSI).[9] All the minimum inhibitory concentration (MIC) values fell below the corresponding cut-off values, therefore, the strain is considered susceptible to the relevant antibiotics.[10]

The genome was interrogated for the presence of acquired antimicrobial resistance genes [redacted][11] No hits were identified.

### 3.1.2. Manufacturing process and characterisation of the additive

The strain is grown on a typical industrial medium, then concentrated by centrifugation and spray-dried.[12] Counts are made at this stage and the spray dried material examined for the presence of microbial and chemical contaminants. [redacted] a minimum specified count of $2 \times 10^{10}$ CFU/g additive. Compliance with this specification was demonstrated in six batches of the additive (mean count $3.5 \times 10^{10}$ CFU/g, range $3.2–4.3 \times 10^{10}$ CFU/g).[14]

Specifications are set for *Salmonella* spp. (absent in 25 g), Enterobacteriaceae ($< 10^3$ CFU/g), yeasts and filamentous fungi ($< 10^2$ CFU/g), *Bacillus cereus* ($< 10^2$ CFU/g), aflatoxin B1 ($< 5$ μg/kg), ochratoxin A ($< 1$ μg/kg), lead ($< 10$ mg/kg), mercury ($< 0.2$ mg/kg), cadmium ($< 5$ mg/kg) and arsenic ($< 4$ mg/kg). Analysis of three batches of the additive showed levels below the corresponding limits of detection,[15] except for lead (0.72−0.74 mg/kg) and yeasts and filamentous fungi (50 CFU/g in one sample) and therefore compliant with the specifications set.[16]

The product is a free-flowing powder. Particle size distribution using laser diffraction[17] and dusting potential by the Stauber–Heubach method were measured in three batches of additive.[18] The results showed that 25% (v/v) of particles had diameters < 50 μm and 7% < 10 μm and the dusting potential was in the range 1.9–3.1 g/m$^3$. The particle size distribution of the dust collected during the dusting

[6] Technical dossier/Section II/Annex II.10.
[7] Technical dossier/Section II/Annexes II.9.
[8] Technical dossier/Section II/Annexes II.13.
[9] Technical dossier/Section II.
[10] Technical dossier/Section II/Annexes II.1.
[11] Technical dossier/Section II/Annex II.9.
[12] Technical dossier/Section II/Annexes II.14, 15 and 16.
[13] Currently under re-evaluation.
[14] Technical dossier/Section II/Annex II.2.
[15] Limits of detection: Enterobacteriaceae (10 CFU/g), yeasts and filamentous fungi (10 CFU/g), *Bacillus cereus* (10 CFU/g), aflatoxin B1 (0.3 μg/kg), ochratoxin A (0.5 μg/kg), mercury (0.02 mg/kg), cadmium (0.2 mg/kg) arsenic (0.5 μg/kg).
[16] Technical dossier/Section II/Annex II.3 and 4.
[17] Technical dossier/Section II/Annex II 6.
[18] Technical dossier/Section II/Annex II.7.

potential measurement was analysed using the laser-diffraction method; all particles showed diameters < 100 μm and approximately 69% (v/v) of particles had a diameter < 10 μm, while the median diameter was approximately 6 μm.

### 3.1.3. Stability and homogeneity

The shelf-life of the additive was determined by monitoring six batches stored in sealed bottles at 25°C for a period of 18 months.[19] No reduction in total *Bacillus* counts was observed during this period.

Four batches of the additive were individually mixed into a commercial vitamin–mineral premixture[20] at a level of $2 \times 10^{11}$ CFU/kg, one batch into a mash feed[21] at a concentration of $1 \times 10^9$ CFU/kg and two batches into pelleted feed[22] for piglets, at a concentration of $1 \times 10^9$ CFU/kg. Samples were stored in sealed containers at 25°C for 3 months (for the premixtures) and 6 months (for feeds). At the end of these periods, counts showed that numbers of bacilli were within ± 0.5 $\log_{10}$ CFU/g of the time zero count in all cases, and therefore stable.

To test the effect of pelleting, one batch of the additive was incorporated into a typical mash feed for pigs (wheat, maize and barley) and the feed was subject to pelleting at three different conditions (80, 90 and 100°C for 8 s).[23] The effect of pelleting itself, obtained by comparison of the initial bacilli counts of the mash and pelleted feed, was small and less than 0.5 $\log_{10}$ difference at either conditions.

To test the capacity of the additive to homogeneously distribute in feed, a total of 10 subsamples were taken from the mash[24] and pelleted[25] feed after mixing with a single batch of additive and analysed for total bacilli counts. Based on the 10 samples, the coefficients of variation were 6% and 5%, respectively.

The results obtained above are considered to apply also to feed and premixtures for poultry.

### 3.1.4. Conditions of use

TechnoSpore® is intended for use in feeds for piglets (suckling and weaned), other growing Suidae, chickens for fattening, other poultry for fattening and ornamental birds at the proposed minimum concentration of $1 \times 10^9$ CFU/kg complete feed.

The applicant is requesting the simultaneous use in feeds for poultry with the authorised coccidiostats monensin sodium, decoquinate, robenidine hydrochloride, lasalocid sodium, halofuginone, narasin, salinomycin sodium, maduramicin ammonium, diclazuril, nicarbazin and nicarbazin/narasin.

## 3.2. Safety

### 3.2.1. Safety for the target species, consumer and the environment

The bacterial species *B. coagulans* is considered by EFSA to be suitable for the qualified presumption of safety approach to safety assessment (EFSA, 2007; EFSA BIOHAZ Panel, 2020). This approach requires the identity of the strain to be conclusively established and evidence that the strain lacks toxigenic potential and does not show acquired antimicrobial resistance determinants for antibiotics of human and veterinary importance. In the view of the FEEDAP Panel, the identity of the active agent is established and the lack of toxigenic potential confirmed. *B. coagulans* DSM 32016 does not show acquired antimicrobial resistance determinants for antibiotics of human and veterinary importance and, therefore, is presumed safe for the target species, consumers of products derived from animals fed the additive and the environment. Since the other components of the additive do not give rise to concerns, TechnoSpore® is also considered safe for the target species, consumer and the environment.

[19] Technical dossier/Section II/Annexes II.17 and 18.
[20] Technical dossier/Section II/Annexes II.19 and 20.
[21] Technical dossier/Section II/Annexes II.21 and 22.
[22] Technical dossier/Section II/Annex II.24 and 25.
[23] Technical dossier/Section II/Annex II.23.
[24] Technical dossier/Section II/Annex II.26.
[25] Technical dossier/Section II/Annex II.27.

### 3.2.2. Safety for the user

The skin[26] and eye[27] irritation potential of TechnoSpore® was tested in a valid study performed according to OECD guidelines 404 and 405, respectively. Results showed that the additive is not a skin or eye irritant.

In a skin sensitisation study following OECD guideline 406, TechnoSpore® did not show any skin sensitisation potential.[28]

Owing to the proteinaceous nature of the active agent, the additive should be considered a respiratory sensitiser. The dusting potential makes it likely that the users will be exposed by inhalation.

#### 3.2.2.1. Conclusions on safety for the user

TechnoSpore® is not a skin/eye irritant or a skin sensitiser but is a respiratory sensitiser.

## 3.3. Efficacy

### 3.3.1. Efficacy for piglets

A total of three trials with weaned piglets, done in three Member States and sharing a common design, was submitted. The details on the study designs are provided in Table 1 and the main results in Table 2.

[redacted]

[26] Technical dossier/Section III/Annex III.7.
[27] Technical dossier/Section III/Annex III.8.
[28] Technical dossier/Section III/Annex III.9.
[29] Technical dossier/Section IV/Annex IV.1 and Supplementary information November 2019/Annex SIn_IV_1f.
[30] Technical dossier/Section IV/Annex IV.2.
[31] Technical dossier/Section IV/Annex IV.3 and Supplementary information November 2019/Annex SIn_IV_3h.

**Table 2:** Effects of TechnoSpore® on the performance of weaned piglets

| Trial | Total bacilli (CFU/kg feed) | Feed intake(1) | Initial body weight (kg) | Final body weight (kg) | Weight gain(2) | Feed to gain ratio | Mortality/ culling (n) |
|---|---|---|---|---|---|---|---|
| 1 | 0 | 31.7 | 7.1 | 28.4 | $21.3^{b}$ | $1.49^{a}$ | 0/0 |
| | $1 \times 10^9$ | 31.0 | 7.0 | 29.1 | $22.1^{a}$ | $1.40^{b}$ | 0/0 |
| 2 | 0 | 0.66 | 7.3 | $23.7^{b}$ | $0.384^{b}$ | $1.71^{a}$ | 0/2 |
| | $1 \times 10^9$ | 0.69 | 7.4 | $25.2^{a}$ | $0.421^{a}$ | $1.63^{b}$ | 1/1 |
| 3 | 0 | 0.48 | 6.7 | $19.7^{b}$ | $0.312^{b}$ | $1.54^{a}$ | 3/2 |
| | $1 \times 10^9$ | 0.50 | 6.7 | $20.7^{a}$ | $0.335^{a}$ | $1.49^{b}$ | 2/3 |

CFU: colony forming unit.
a,b: Mean values within a trial and within a column with a different superscript are significantly different ($p < 0.05$).
(1): Total feed intake in trial 1 and daily feed intake in trials 2 and 3.
(2): Total weight gain in trial 1 and average daily gain in trials 2 and 3.

Piglets that received TechnoSpore® at the recommended level showed a better weight gain and feed to gain ratio in three studies and increased the final body weight in two of them.

### 3.3.2. Efficacy for chickens for fattening

A total of four trials in chickens for fattening, conducted in three Member States, were submitted. The details on the study designs are provided in Table 3 and the main results in Table 4.

[redacted]

[redacted]

[32] Technical dossier/Section IV/Annex IV.4 and Supplementary information November 2019/AnnexSIn_IV_4g.
[33] Technical dossier/Section IV/Annex IV.5 and Supplementary information November 2019/AnnexSIn_IV_5g.
[34] Technical dossier/Section IV/Annex IV.6 and Supplementary information November 2019/AnnexSIn_IV_6g.
[35] Technical dossier/Section IV/Annex IV.7 and Supplementary information November 2019/AnnexSIn_IV_7g.

**Table 4:** Effects of TechnoSpore® on the performance of chickens for fattening

| Trial | Total bacilli (CFU/kg feed) | Feed intake(1) (g or kg) | Final body weight (g) | Weight gain(2) (g) | Feed to gain ratio | Mortality/culling (%) |
|---|---|---|---|---|---|---|
| 1 | 0 | n.r. | 1,897[b] | 1,854 | 1.87 | 0.9/1.0 |
| | $1 \times 10^9$ | | 1,926[a] | 1,883 | 1.83 | 1.4/1.7 |
| 2 | 0 | 126.1[b] | 2,519[b] | 71.0 | 1.78 | 1.2/1.2 |
| | $1 \times 10^9$ | 129.3[a] | 2,583[a] | 72.5 | 1.78 | 1.7/1.7 |
| 3 | 0 | 3,231 | 2,009[b] | 1,967[b] | 1.64 | 0.9/0.6 |
| | $1 \times 10^9$ | 3,293 | 2,057[a] | 2,015[a] | 1.63 | 0.6/0.3 |
| 4 | 0 | 668.7[b] | 2,508[b] | 58.9 | 1.98 | 3.9/2.1 |
| | $1 \times 10^9$ | 693.1[a] | 2,555[a] | 59.9 | 2.01 | 3.5/1.8 |

CFU: colony forming unit; n.r.: not reported.
a,b: Mean values within a trial and within a column with a different superscript are significantly different $p < 0.05$.
(1): Total feed intake per pen (kg) in trial 4, average daily feed intake per bird (g) in trial 2 and total feed intake per bird (g) in trial 3.
(2): Total weight gain in trials 1 and 3 and average daily weight gain in trials 2 and 4.

Total feed intake for the whole experimental period was not reported or statistically analysed in trial 1. Therefore, this study can only used as supportive evidence. Chickens receiving TechnoSpore® at the recommended level of $1 \times 10^9$ CFU/kg complete feed showed a greater feed intake in two studies, weight gain in one study and final weight compared to the control in the three studies considered.

### 3.3.3. Compatibility with coccidiostats

To support the compatibility of *B. coagulans* DSM 32016 with monensin sodium, decoquinate, robenidine hydrochloride, lasalocid sodium, halofuginone, narasin, salinomycin sodium, maduramicin ammonium, diclazuril, nicarbazin and a mixture (1:1) nicarbazin/narasin, one *in vitro*[36] and one *in vivo*[37] studies have been submitted.

In the *in vitro* trial, [redacted] for halofuginone and diclazuril [redacted] *B. coagulans* DSM 32016 from TechnoSpore® is considered compatible with these two coccidiostats. [redacted]

The scope of the *in vivo* study was to compare the performance and caecal counts of chickens receiving simultaneously Technospore® and one of nine coccidiostats in the feed (9 pens, each receiving one of the following coccidiostats: monensin sodium, decoquinate, robenidine hydrochloride, lasalocid sodium, narasin, salinomycin sodium, maduramicin ammonium, diclazuril, nicarbazin/narasin and semduramycin) at approximately the maximum authorised level in feed for chickens. Since diclazuril showed no inhibition in the *in vitro* test described above, this group was considered by the applicant as the control. However, since a proper control in which animals receiving the additive without coccidiostat was not included, no conclusion can be drawn on the compatibility of *B. coagulans* DSM 32016 from TechnoSpore® with these coccidiostats.

### 3.3.4. Conclusions on efficacy

Based on the results of the studies submitted by the applicant the Panel concludes that Technospore® has the potential to be efficacious as a zootechnical additive in weaned piglets and chickens for fattening at $1 \times 10^9$ CFU/kg complete feed. This conclusion is extended to suckling piglets for the period in which solid feed is given to the animals.

No specific data were submitted for other Suidae, other birds for fattening or ornamental birds. However, the effects of Technospore® can be reasonably assumed to be the same within the species of Suidae and within the avian species. The conclusions drawn from the studies in weaned piglets can be extrapolated to other Suidae in the suckling and weaning phases at the same inclusion level.

[36] Technical dossier/Section II/Annex II.28.
[37] Technical dossier/Section II/Annexes II.30–33.

Similarly, the conclusions from chickens for fattening can be extrapolated to other poultry for fattening and ornamental birds.

Considering the data submitted, the FEEDAP Panel concludes that *B. coagulans* DSM 32016 included in Technospore® is compatible with halofuginone and diclazuril. Based on the data provided, no conclusion can be drawn on the compatibility of the additive with monensin sodium, decoquinate, robenidine hydrochloride, lasalocid sodium, narasin, salinomycin sodium, maduramicin ammonium, nicarbazin and nicarbazin/narasin.

### 3.4. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[38] and Good Manufacturing Practice.

## 4. Conclusions

The active agent comprising the additive (*B. coagulans* DSM 32016) meets the requirements of the qualified presumption of safety (QPS) approach to safety assessment and is presumed safe for the target animals, consumers of products derived from animals fed the additive and the environment. Since the other components of the additive do not rise concerns, TechnoSpore® is also considered safe for the target species, consumer and the environment.

TechnoSpore® is not a skin/eye irritant or a skin sensitiser but should be considered a respiratory sensitiser.

TechnoSpore® has the potential to be efficacious as a zootechnical additive in weaned piglets and chickens for fattening at $1 \times 10^9$ CFU/kg complete feed. This conclusion is extended to suckling piglets and extrapolated to other growing *Suidae* at the same physiological stage and to other birds for fattening and ornamental birds at the same use level.

The FEEDAP Panel concludes that *B. coagulans* DSM 32016 included in Technospore® is compatible with halofuginone and diclazuril but no conclusions can be drawn on the compatibility of the additive with monensin sodium, decoquinate, robenidine hydrochloride, lasalocid sodium, narasin, salinomycin sodium, maduramicin ammonium, nicarbazin and narasin/nicarbazin.

## 5. Documentation as provided to EFSA/Chronology

| Date | Event |
|---|---|
| 11/04/2019 | Dossier received by EFSA. TechnoSpore® (Bacillus coagulans DSM 32016). Submitted by Biochem Zusatzstoffe Handels- und Produktionsges. mbH |
| 06/05/2019 | Reception mandate from the European Commission |
| 31/07/2019 | Application validated by EFSA – Start of the scientific assessment |
| 26/09/2019 | Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterization and efficacy* |
| 26/11/2019 | Reception of supplementary information from the applicant - Scientific assessment re-started |
| 31/10/2019 | Comments received from Member States |
| 07/11/2019 | Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives |
| 25/05/2020 | Opinion adopted by the FEEDAP Panel. End of the Scientific assessment |

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[38] Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

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## Abbreviations

| | |
|---|---|
| CFU | colony forming unit |
| CLSI | Clinical and Laboratory Standards Institute |
| EURL | European Union Reference Laboratory |
| FEEDAP | EFSA Panel on Additives and Products or Substances used in Animal Feed |
| MIC | minimum inhibitory concentration |
| PFGE | pulsed field gel electrophoresis |
| QPS | qualified presumption of safety |
| RH | relative humidity |

# Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for TechnoSpore®

In the current application authorisation is sought under Article 4(1) for *Bacillus coagulans* DSM 32016 under the category/functional group 4(b) 'zootechnical additives'/'gut flora stabilisers', according to Annex I of Regulation (EC) No 1831/2003. The authorisation is sought for the use of the *feed additive* for piglets (suckling and weaned), other growing *Suidae*, chickens for fattening, other poultry for fattening and ornamental birds.

According to the Applicant, the *feed additive* contains viable spores of the non-genetically modified strain *Bacillus coagulans* DSM 32016 as an active substance. The *feed additive* is to be marketed as a powder (under the trade name - *TechnoSpore®*) containing a minimum content of the active substance of $2 \times 10^{10}$ Colony Forming Unit (CFU)/g TechnoSpore®. The *feed additive* is intended to be used in *feedingstuffs* and *premixtures* at a minimum dose of $1 \times 10^{9}$ CFU/kg complete feedingstuffs.

For the identification of *Bacillus coagulans* DSM 32016, the EURL recommends for the official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised methodology for the genetic identification of bacterial strains.

For the enumeration of *Bacillus coagulans* DSM 32016 in the *feed additive, premixtures* and *feedingstuffs* the Applicant submitted the slightly modified method based on ring-trial validated spread plate method EN 15787, where Tween® 80 is added at 1% in buffer and diluent in the case of all matrices and where the final dilution is heated. Based on the performance characteristics and experimental data available, the EURL recommends the modified method EN 15787 for the official control for the enumeration of *Bacillus coagulans* DSM 32016 in the *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), as last amended by Regulation (EU) 2015/1761) is not considered necessary.