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Safety and efficacy of Correlink™ ABS747 *Bacillus subtilis* (*Bacillus velezensis* NRRL B-67257) as a feed additive for all growing poultry species

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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 Correlink™ ABS747 *Bacillus subtilis* (hereafter designated as Correlink™ ABS747) when used as a feed additive for chickens for fattening, turkeys for fattening, chickens reared for laying, turkeys reared for breeding and minor growing poultry species. The product under assessment is based on viable spores of a strain originally identified as *Bacillus subtilis*, which in the course of the current assessment, was reclassified as *Bacillus velezensis* NRRL B-67257. The bacterial species *B. velezensis* is considered suitable for the qualified presumption of safety (QPS) approach to safety assessment. The identity of the active agent was established and it does not harbour acquired antimicrobial resistance genes, lacks toxigenic potential and does not have the capacity to produce aminoglycosides. Following the QPS approach to safety assessment, *B. velezensis* NRRL B-67257 is presumed safe for the target species, consumers of products derived from animals fed the additive and the environment. Since no concerns are expected from the other components of the additive, Correlink™ ABS747 is also considered safe for the target species, consumers of products derived from animals fed the additive and the environment. Correlink™ ABS747 is not irritant to skin and eyes or a skin sensitiser but is a respiratory sensitiser. The Panel is not in the position to conclude on the efficacy of Correlink™ ABS747 for the target species due to lack of data.

Keywords: Zootechnical additive, Correlink™ ABS747, *Bacillus velezensis* NRRL B-67257, safety, QPS, efficacy

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Question number: EFSA-Q-2019-00803

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1. Introduction

1.1. Background and Terms Reference

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 Elanco GmbH[2] for authorisation of the product Correlink™ ABS747 *Bacillus subtilis* (*Bacillus velezensis* NRRL B-67257[3]), when used as a feed additive for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding and minor growing poultry species (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 4 February 2020.

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 Correlink™ ABS747 *Bacillus subtilis* (*Bacillus velezensis* NRRL B-67257), when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

The additive Correlink™ ABS747 *Bacillus subtilis* (hereafter referred to as Correlink™ ABS747) is a preparation containing viable spores of *Bacillus velezensis* NRRL B-67257. It has not been previously authorised in the European Union.

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[4] in support of the authorisation request for the use of Correlink™ ABS747 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.[5]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Correlink™ ABS747 is in line with the principles laid down in Regulation (EC) No 429/2008[6] 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 assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018a),

[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] Elanco Animal Health, 201 South Main St, 08520, Highstown, New Jersey, US represented by Elanco GmbH, Heinz-Lohmann Str4, 27472 Cuxhaven, Germany.
[3] Originally designated as *Bacillus subtilis* NRRL B-67257.
[4] FEED dossier reference: FAD-2019-0074.
[5] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2019-0074_correlink.pdf
[6] 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.

Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018b) and Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019).

3. Assessment

Correlink™ ABS747 is a preparation of viable spores of a single strain of *B. velezensis* intended for use as a zootechnical additive (functional group: gut flora stabiliser) in feed for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding and minor growing poultry species.

3.1. Characterisation

3.1.1. Characterisation of the additive

The active agent of Correlink™ ABS747 is a strain of unknown origin deposited at the Agricultural Research Culture Collection (NRRL) with the accession number NRRL B-67257.[7] The strain has not been genetically modified.

The full genome of the active agent was sequenced and used for identification purposes. The taxonomic identification as *B. velezensis* was established by digital DNA–DNA hybridisation (dDDH) against main genomes of type strains of *Bacillus* species. The dDDH values showed 80.4% and 80.1% similarity compared to the type strains *B. amyloliquefaciens* subsp. *plantarum* FZB42^T (synonym *B. velezensis*) and *B. velezensis* NRRL B-41580^T, respectively.[8]

The toxigenic potential of *B. velezensis* NRRL B-67257 was assessed according to the Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018a,b).[9] No lysis of Vero cells was detected, therefore, the strain is considered to be non-toxigenic.

The susceptibility of the strain to the antibiotics recommended by the FEEDAP Panel was tested by broth microdilution following the FEEDAP guidance (EFSA FEEDAP Panel, 2018a,b).[10] All the minimum inhibitory concentration (MIC) values determined were equal or fell below the corresponding cut-off values. Therefore, *B. velezensis* NRRL B-67257 is susceptible to the relevant antibiotics.

The whole genome sequence (WGS) of the active agent was interrogated for the presence of antimicrobial resistance (AMR) genes against the CARD (criteria: perfect, strict & loose, complete genes only, 95% identity nudge used) and the ResFinder databases (thresholds: 90% identity and 60% coverage).[11] The Panel notes that the criteria applied in the last search were less stringent than the recommended. However, considering that neither search evidenced any hit of concern and that the strain proved susceptible to all the relevant antibiotics tested, it can be concluded that *B. velezensis* NRRL B-67257 does not harbour acquired AMR genes.

Similarly, the WGS was interrogated for the presence of virulence factors by comparing the genome sequences of NRRL B-67257 against the translated sequences of *Bacillus cereus* ATCC 14579^T using tBLASTn (thresholds: 90% identity and 60% coverage).[12] The search did not evidence any hit. Therefore, the strain lacks the *B. cereus* virulence factors.

To exclude the capacity of the active agent to produce aminoglycosides, the supernatants from the culture of three batches of the additive were tested against reference strains (*Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 11229, *Bacillus cereus* ATCC 2, *Bacillus circulans* ATCC 4516, *Streptococcus pyogenes* ATCC 12344 and *Serratia marcescens* ATCC 14041).[13] No inhibition was observed, denoting the lack of antimicrobial production, including aminoglycosides.

[7] Technical dossier/Section II/Annex.II.2.1.2.1.
[8] Technical dossier/Section II/Annexes.II.2.1.2.2 and II.2.1.2.3 and Supplementary information May 2020/Annexes II.2.1.2.6, II.2.1.6.1.747, II.2.1.6.2.747 and II.2.1.6.3.747.
[9] Technical dossier/Section II/Annex II.2.2.2.3.
[10] Technical dossier/Section II/Annex II.2.2.2.2.
[11] Technical dossier/Section II/Annex.II.2.1.2.3 and Supplementary information May 2020/Annexes II.2.1.2.6, II.2.1.6.1.747, II.2.1.6.2.747 and II.2.1.6.3.747.
[12] Technical dossier/Section II/Annex.II.2.1.2.3.
[13] Technical dossier/Section II/Annex.II.2.2.2.1.

3.1.2. Manufacturing process and characterisation of the additive

Viable spores of *B. velezensis* NRRL B-67257 are produced by fermentation, [redacted].[14] The final additive is then produced and standardised [redacted] to reach a minimum guaranteed concentration of 1×10^{11} colony forming units (CFU) per gram of additive.[15] Compliance with this specification was demonstrated in five batches of the additive (mean count 1.6×10^{11} CFU/g, range $1.2–1.9 \times 10^{11}$ CFU/g).[16]

Three batches of the additive were analysed for the presence of chemical and microbiological contaminants.[17] Values for lead were 0.05–0.15 mg/kg, for dioxins and the sum of dioxins and dioxin-like PCBs were 0.01–0.08 ng WHO-PCDD/F-TEQ/kg and 0.01–0.09 ng WHO-PCDD/F-PCB-TEQ/kg, respectively, while those for cadmium, mercury, arsenic and mycotoxins were below the respective limit of detection (LOD).[18] Similarly, counts of *Bacillus cereus, Enterobacteriaceae,* yeasts and filamentous fungi were also below the LOD and *Salmonella* spp. was absent in 25 g.[19]

Correlink™ ABS747 is a dry preparation. The dusting potential of three batches of the additive, tested using the Stauber–Heubach method showed values of 0.11–1.14 g/m^3.[20] The particle size of the additive in the same three batches of the additive, measured by laser diffraction, showed that approximately 29–39% of the particles have diameters < 50 μm and 5–7% of the particles have diameters < 10 μm.

3.1.3. Stability and homogeneity

The shelf-life of the additive (1.75×10^{11} CFU/g) was determined by monitoring three batches stored in the original packaging (aluminium foil laminate bags) at 25°C/60% relative humidity (RH) and 30°C/65% RH for a period of 24 months and at 40°C/75% RH for a period of 18 months.[21] No reduction in total bacilli counts (< 0.5 log) was observed at any condition during the experimental period.

Three batches of the additive were individually mixed into a standard chicken vitamin/mineral premixture (containing 220 mg choline chloride/kg) at the proposed conditions of use and samples were stored for 6 months at three conditions (25°C/60% RH, 30°C/65% RH and 40°C/ambient RH).[22] The results after 6 months showed no reduction in bacilli counts at any tested condition.

To investigate the stability of the additive during pelleting, samples from three batches of the additive were incorporated into a typical mash feed for chickens (maize and soybean meal) at a concentration of 1.5×10^8 CFU/kg feed and subjected to pelleting at two different pelleting conditions (80–85°C and 90–95°C).[23] The bacilli counts were determined before and after the pelleting process; at the end, values were < 0.5 log than initial counts. To investigate the stability of the additive in complete feed, subsamples of the samples described above were collected before and after pelleting, and stored for up to 3 months at 25°C/60% RH% and 40°C/ambient RH. No reduction in bacilli counts (< 0.5 log) was seen in either the mash or pelleted feed at any conditions after 3 months storage.

To examine the capacity of homogenous distribution of the additive in feed, a total of ten sub-samples were taken from the mash feed[23] and premixture[22] described above analysed for total bacilli counts. The coefficient of variation was < 1% for both mash feed and premixture.

3.1.4. Conditions of use

Correlink™ ABS747 is intended to be used in feed for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding and minor growing poultry species at the minimum concentration of 1.5×10^8 CFU/kg complete feedingstuffs.

[14] Technical dossier/Section II/Manufacturing process.
[15] Technical dossier/Section II/Annex.II.2.1.3.1.
[16] Technical dossier/Section II/Annex.II.1.4.3.
[17] Technical dossier/Section II.1.4.3.
[18] LODs: arsenic: 0.5 mg/kg, cadmium: 0.25 mg/kg, mercury: 0.10 mg/kg, aflatoxin B1: 1.3 ppb, aflatoxin B2: 1.2 ppb, aflatoxin G1: 1.1 ppb, aflatoxin G2: 1.6 ppb, zearalenone: 51.7 ppb, ochratoxin: 1.1 ppb, T2 and HT-2 toxins: 0.2 ppm, deoxynivalenol, fumonisins B1, B2 and B3: 0.1 ppm.
[19] *B. cereus* < 100 CFU/g, Enterobacteriaceae < 10 MPN/g, *Salmonella* spp. absent in 25 g, yeast and filamentous fungi < 10 CFU/g.
[20] Technical dossier/Section II/Annex.II.1.5.1.
[21] Technical dossier/Section II/Annex_II.4.1.1.
[22] Technical dossier/Section II/Annex_II_4.1.3.
[23] Technical dossier/Section II/Annex_II.4.1.2.

The applicant is also asking for the authorisation to use the additive simultaneously with the authorised coccidiostats: diclazuril, decoquinate, halofuginone, monensin, salinomycin, narasin, robenidine, maduramicin and lasalocid.

3.2. Safety

3.2.1. Safety for target animals, consumers and environment

The bacterial species *B. velezensis* 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 provided that it does not harbour acquired antimicrobial resistance genes, that it lacks toxigenic potential and it does not have the capacity to produce aminoglycosides. In the view of the FEEDAP Panel, the identity of the active agent as *B. velezensis* was established and the compliance with the other qualifications confirmed. Therefore, the FEEDAP Panel concludes that the *B. velezensis* NRRL B-67257 does not raise safety concerns for the target species, consumers of products derived from animals fed the additive and the environment. Since no concerns are expected from the other components of the additive, Correlink™ ABS747 is also considered safe for the target species, consumers of products derived from animals fed the additive and the environment.

3.2.2. Safety for user

The skin[24] and eye[25] irritation potential of Correlink™ ABS747 was tested in valid studies performed according to the OECD guideline 404 and 405, respectively. The results showed that the additive is not a skin nor eye irritant.

In a valid skin sensitisation study following OECD guideline 406, Correlink™ ABS747 did not show any skin sensitisation potential.[26]

The dusting potential (up to 1.14 g/m^3) and the particle size distribution tested indicate that exposure by inhalation cannot be excluded. Owing to the proteinaceous nature of the active agent, Correlink™ ABS747 is considered a respiratory sensitiser.

3.2.2.1. Conclusions on safety for the user

Correlink™ ABS747 is not irritant to skin and eyes nor a skin sensitiser. Owing to the proteinaceous nature of the active agent, Correlink™ ABS747 should be considered a respiratory sensitiser.

3.3. Efficacy

3.3.1. Efficacy for chickens for fattening

A total of three trials conducted in chickens for fattening were submitted. However, two of them were not further considered due to the contamination of the control diets with the additive under assessment. In the first case[27] the total bacilli counts in the starter, grower and finisher diets of both the control and treated groups were equivalent (i.e. control: 0.7/0.4/0.9 $\times$ 10^8 CFU/kg feedingstuffs vs Correlink™ ABS747: 1.2/1.0/0.8 $\times$ 10^8 CFU/kg feedingstuffs). The presence of the active agent in all diets was confirmed by analysis using, according to the applicant, a strain-specific polymerase chain reaction (PCR) targeting a functional gene of *B. velezensis* NRRL B-67257. Therefore, it can be inferred that the control diets were contaminated with the additive. In the second case,[28] the total bacilli counts in the diets of both the control and treated groups were also equivalent (i.e. control: 0.8/1.6/1 $\times$ 10^8 CFU/kg feedingstuffs vs Correlink™ ABS747: 2.8/4.5/1.9 $\times$ 10^8 CFU/kg feedingstuffs), but the presence of *B. velezensis* NRRL B-67257 could not be confirmed due the non-availability of samples. However, considering the similarities between the trials (i.e. they were both ran in the same trial site and with the same design), and that *B. velezensis* NRRL B-67257 was identified using the method mentioned above in faecal samples collected from both treatment groups, it cannot be excluded that also in this case the control diets were contaminated with the additive.

[24] Technical dossier/Section III/Anne×_III_3.1.2.1.
[25] Technical dossier/Section III/Annex III.3.1.2.2.
[26] Technical dossier/Section III/Annex III.3.1.2.3.
[27] Technical dossier/Section IV/Annex IV.3.3 and Supplementary information May 2020/Annexes IV.3.3.1 and IV.3.3.2.
[28] Technical dossier/Section IV/Annex IV.3.2 and Supplementary information May 2020/Annex IV.3.2.1.

In the only trial considered, 576 one-day-old male chickens (Ross 308) were fed either a non-supplemented diet (control) or a diet containing Correlink™ ABS747 at 1.5×10^8 CFU/kg complete feed (confirmed by analysis, Table 1).[29] The wheat, soybean meal and barley-based diets in mash form were administered for 42 days. The health and mortality of birds were monitored throughout the study and the body weight and feed intake were recorded. Feed to gain ratio was calculated. The data were analysed with an analysis of variance and group means were compared with a two-sided t-test. The pen was the experimental unit for all parameters. The significance level was set at $p < 0.05$. Results are presented in Table 2.

Table 1: Trial design and dosages of the efficacy trial performed in chickens for fattening

Total animals (animals per replicate) Replicates per treatment	Breed sex (duration)	Composition feed (Form)	Treatment groups	Analysed bacilli counts (CFU/kg feed)
576 (12) 24	Ross 308 Males (42 days)	Wheat, soya bean meal, barley (Mash)	Control Correlink™ ABS747	$2.7/3.4/3.2 \times 10^6$ $1.5/1.6/1.6 \times 10^8$

CFU: colony forming unit.

Table 2: Effects of Correlink™ ABS747 on the performance of chickens for fattening

Treatment groups	Daily feed intake (g)	Final body weight (kg)	Average daily weight gain (g)	Feed to gain ratio	Mortality and culling (%)
Control	115.8^b	2.95^b	69.1^b	1.62	4.2
Correlink™ABS747	120.5^a	3.13^a	73.6^a	1.62	1.7

^{a,b}: Within a column mean values with a different superscript are significantly different $p < 0.05$.

The chickens in the Correlink™ ABS747 group showed a significantly greater final weight and weight gain compared to control animals in the only trial considered. However, there are insufficient data to allow the Panel to conclude on the efficacy of Correlink™ ABS747 for chickens for fattening.

3.3.2. Compatibility with coccidiostats

An *in vitro* study was conducted to support the compatibility of *B. velezensis* NRRL B-67257 with diclazuril, decoquinate, halofuginone, monensin, salinomycin, narasin, robenidine, maduramicin and lasalocid.[30] The MIC values were measured using the broth microdilution method and showed values for diclazuril (> 4.8 mg/L), decoquinate (> 160 mg/L) and halofuginone (> 12 mg/L) greater than four times their maximum authorised levels in feed (1.2, 40 and 3 mg/kg, respectively). Therefore, *B. velezensis* NRRL B-67257 is compatible with diclazuril, decoquinate and halofuginone. The MIC values for the remaining coccidiostats (monensin and lasalocid: < 31.25 mg/L, salynomicin and narasin: < 17.5 mg/L, robenidine < 9 mg/L, maduramicin: 6) were below four times the corresponding maximum authorised concentrations in feed. Therefore, a demonstration of compatibility *in vivo* would be needed. In the absence of such studies, the FEEDAP Panel is not in the position to conclude on the compatibility of *B. velezensis* NRRL B-67257 with monensin, lasalocid salinomycin, narasin, robenidine and maduramicin.

3.3.3. Conclusions on efficacy for the target species

The Panel is not in the position to conclude on the efficacy of Correlink™ ABS747 for chickens for fattening, turkeys for fattening, chickens reared for laying, turkeys reared for breeding or minor growing poultry species due to lack of data.

B. velezensis NRRL B-67257 is compatible with diclazuril, decoquinate and halofuginone. No conclusions can be drawn on the compatibility of the additive with monensin, salinomycin, narasin, robenidine, maduramicin and lasalocid.

[29] Technical dossier/Section IV/Annex IV.3.1.
[30] Technical dossier/Section II/Annex_II.4.4.1

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[31] and Good Manufacturing Practice.

4. Conclusions

Correlink™ ABS747 is considered safe for the target species, consumers of products derived from animals fed the additive and the environment.

Correlink™ ABS747 is not irritant to skin and eyes or a skin sensitiser but is a respiratory sensitiser.

The Panel is not in the position to conclude on the efficacy of Correlink™ ABS747 for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding or minor growing poultry species due to lack of data.

Bacillus velezensis NRRL B-67257 is compatible with diclazuril, decoquinate and halofuginone. No conclusions can be drawn on the compatibility of the additive with monensin, salinomycin, narasin, robenidine, maduramicin and lasalocid.

5. Documentation as provided to EFSA/Chronology

Date	Event
06/05/2019	Reception mandate from the European Commission
18/11/2019	Dossier received by EFSA. Correlink™ (*Bacillus velezensis* NRRL B-67257) as a feed additive for all growing poultry species. Submitted by Elanco GmbH
04/02/2020	Application validated by EFSA – Start of the scientific assessment
17/03/2020	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, efficacy*
21/04/2020	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
04/05/2020	Comments received from Member States
25/05/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
30/09/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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[31] 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

AMR	antimicrobial resistance
BW	body weight
CFU	colony forming unit
dDDH	digital DNA–DNA hybridisation
EURL	European Union Reference Laboratory
LOD	limit of detection
MIC	minimum inhibitory concentration
OECD	Organisation for Economic Co-operation and Development
PCB	polychlorinated biphenyl
PCDD/F	polychlorinated dibenzo-p-dioxin and dibenzofuran
PCR	polymerase chain reaction
QPS	qualified presumption of safety
RH	relative humidity
TEQ	toxic equivalent
WGS	whole genome sequence

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Correlink™ ABS747 *Bacillus subtilis*

In the current application authorisation is sought under Article 4(1) for *Bacillus velezensis*[32] ABS747[33] under the category/functional group 4(b) 'zootechnical additives'/'gut flora stabilisers', according to Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for the use of the *feed additive* for chickens for fattening, turkeys for fattening, chickens reared for laying, turkeys reared for breeding and minor poultry species.

According to the Applicant, the *feed additive* contains as *active substance* viable spores of the non-genetically modified strain *Bacillus subtilis* ABS747. The *feed additive* is to be marketed as a dry preparation containing a minimum content of *active substance* of 1×10^{11} Colony Forming Unit (CFU)/g and to be used directly in *feedingstuffs* or through *premixtures* at a minimum dose of 1×10^{5} CFU/g complete *feedingstuffs*.

For the identification of *Bacillus subtilis* ABS747, 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 *Bacillus subtilis* ABS747 in the *feed additive*, *premixtures* and *feedingstuffs*, the Applicant submitted an in-house plate count method single-laboratory validated and further verified by another laboratory. However, the EURL is aware of a ring-trial validated spread plate CEN method EN 15784 developed to enumerate and differentiate spores of several *Bacillus* spp. Furthermore the Applicant confirmed, upon request of the EURL, the suitability of the CEN method EN 15784 for this product.

Based on the performance characteristics reported and the applicability statement provided by the Applicant, the EURL recommends instead the ring trial validated CEN method EN 15784 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), as last amended by Regulation (EU) 2015/1761) is not considered necessary.

[32] Now designated as *Bacillus velezensis.*
[33] This is an in-house identifier used by the applicant. The correct deposition number is *Bacillus velezensis* NRRL B-67259.