SCIENTIFIC OPINION

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Safety and efficacy of Cinergy® Life B3 HiCon (*Bacillus amyloliquefaciens* NRRL B-50508, *B. amyloliquefaciens* NRRL B-50509 and *Bacillus subtilis NRRL* B-50510) as a feed additive for pigs for fattening and minor porcine species

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Abstract

The additive contains viable spores of two strains of *Bacillus amyloliquefaciens* and a single strain of *Bacillus subtilis* and is intended to be used with pigs for fattening and minor porcine species at a minimum inclusion level of 1.5×10^8 colony forming units (CFU)/kg complete feedingstuffs. The two bacterial species are considered suitable for the qualified presumption of safety (QPS) approach to safety assessment, which requires the identity of the strains to be established and evidence that they lack toxigenic potential and acquired antimicrobial resistance determinants for antibiotics of human and veterinary importance. The identity of the three active agents is established and the lack of toxigenic potential confirmed. The two *B. amyloliquefaciens* strains do not show resistance to relevant antibiotics, and are presumed safe for the target species, consumers and the environment. The *B. subtilis* strain showed a low level of resistance to streptomycin, for which acquired resistance genes were not identified. Therefore, it also complies with the QPS qualifications and is presumed safe for the target species, consumer and the environment. Since no other component give rise to concerns, Cinergy® Life B3 HiCon is also considered safe for the target species, consumers and the environment. In the absence of data, no conclusions can be made on the skin or eye irritancy or the potential for dermal sensitisation of the additive. Owing to the proteinaceous nature of the active agents, the additive is considered a potential respiratory sensitiser. However, the low dusting potential makes it unlikely that additive poses a risk for the respiratory system. Cinergy® Life B3 HiCon showed a potential to improve the feed to gain ratio in pigs for fattening given the additive at a minimum inclusion level of 1.5×10^8 CFU/kg complete feed. This conclusion is extrapolated to minor porcine species at the same application rate and for an equivalent growth phase.

Keywords: Cinergy® Life B3 HiCon, *Bacillus amyloliquefaciens*, *Bacillus subtilis*, pigs for fattening, safety, streptomycin resistance, efficacy

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1. Introduction

1.1. Background and Terms of 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 Cargill Incorporated, represented By Provimi Holding BV[2] for authorisation of the product Cinergy® Life B3 HiCon (*Bacillus amyloliquefaciens* NRRL B-50508, *B. amyloliquefaciens* NRRL B-50509 and *Bacillus subtilis* NRRL B-50510) when used as a feed additive for pigs for fattening and minor porcine species (category: zootechnical additive; functional group: gut flora stabiliser).

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 8 January 2018.

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 Cinergy® Life B3 HiCon (*Bacillus amyloliquefaciens* NRRL B-50508, *B. amyloliquefaciens* NRRL B-50509 and *Bacillus subtilis* NRRL B-50510), when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

The additive Cinergy® Life B3 HiCon 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 active agent as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008[4] and the applicable EFSA guidance documents.

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 Cinergy® Life B3 HiCon is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on zootechnical additives (EFSA FEEDAP Panel, 2012a), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b), Guidance on the assessment of the toxigenic potential of *Bacillus* species used in animal nutrition (EFSA FEEDAP Panel, 2014), Guidance on the assessment of bacterial susceptibility to antimicrobials of

[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] Cargill Incorporated, represented By Provimi Holding BV, Veerlaan 17-23, 3072 AN, Rotterdam, The Netherlands.
[3] FEED dossier reference: FAD-2017-0060.
[4] 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.
[5] The full report is available on the EURL website: https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports/fad-2017-0060?search&form-return

human and veterinary importance (EFSA FEEDAP Panel, 2012c) and Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018).

3 Assessment

The additive is a preparation containing viable spores of two strains of *B. amyloliquefaciens* and a single strain of *B. subtilis* intended to be used in feed for pigs for fattening and minor porcine species as a zootechnical additive to improve performance.

3.1 Characterisation

3.1.1. Characterisation of the active agents

[redacted] All strains are deposited with the United States Department of Agriculture Agricultural Research Culture Collection (NRRL Collection) with the accession numbers NRRL B-50508 and NRRL 50509 for the two *B. amyloliquefaciens* strains and NRRL B-50510 for the *B. subtilis*.[7] None of the strains have been genetically modified.[8] [redacted] Randomly amplified polymorphic DNA (RAPD) PCR genotyping analysis supported the differentiation between the additive strains and between other similar strains.

[redacted] No cytotoxic effects were detected in any of the strains.

[redacted] (Ohmiya et al., 1989; Kunst et al., 1997)

3.1.2. Characterisation of the additive

[redacted]

The final product is a powder with a minimum specified count of *Bacillus* spp. is 2.5×10^9 CFU/g additive with the three strains contributing approximately in equal numbers. Compliance with this

[redacted]

[7] Technical dossier/Section II/ Annex II.2.1.2.3.
[8] Technical dossier/Section II/ Annex II.2.1.2.1.

[redacted]

specification was demonstrated in five batches of the additive. The counts given for the individual strains are the same for all batches [redacted]

Three batches of the additive were analysed for heavy metals and arsenic, dioxins and dioxin-like polychlorinated biphenyls (PCBs) and mycotoxins. Lead, cadmium and arsenic were detected at mean concentrations of 1.8, 0.16 and 0.31 mg/kg additive respectively, while mercury was not detected (limit of quantification (LOQ) < 0.01 mg/kg additive).[16] The mean upper bound value for the sum of dioxins and dioxin-like PCBs was 0.09 ng WHO-PCDD/F-PCB-TEQ/kg additive. These values are substantially below the maximum levels permitted in feed materials and do not raise concerns. None of the mycotoxins included in the analysis could be detected (LOQ total aflatoxins < 0.05 μg/kg, total fumonisins < 30 μg/kg, total HT-2 Toxin < 50 μg/kg, total T-2 toxin < 5 μg/kg zearalenone < 25 μg/kg, ochratoxin < 5 μg/kg and vomitoxin < 50 μg/kg). The same three batches were examined for evidence of microbial contamination. Coliforms, *Escherichia coli*, *Bacillus cereus* and *Staphylococcus aureus* were below the detection limits (< 10 CFU/g additive) and no *Listeria* or *Salmonella* species could be isolated from 25 g or 50 g of the additive, respectively.

Sterile culture filtrate obtained from growth of three batches of the additive did not produce zones of clearing in lawn cultures of a number of test bacterial species indicating that the strains do not produce extracellular antibacterial activity.[17]

Particle size distribution using laser diffraction[18] and dusting potential by the Stauber–Heubach method were measured in three batches of additive. The mean particle size was approximately 430 μm with only around 2% (v/v) of particles with diameters < 100 and 0.7% < 10 μm. The dusting potential reflected both the particle size distribution and the inclusion of an unspecified mineral oil as binder and gave a mean value of 0.2 g/m^3.[19]

3.1.3. Stability and homogeneity

The shelf-life of the additive was determined by monitoring three batches stored at either 25°C/60% relative humidity (RH) or 40°C/75% RH for a period of 24 months.[20] Essentially no reduction in total *Bacillus* count was observed at either temperature.

Three batches of the additive were individually mixed into a commercial vitamin-mineral premix (containing choline chloride) at a concentration of 1×10^{11} CFU/kg premix and samples taken and stored in a sealed container for 6 months at 25°C.[21] Results showed that numbers of bacilli in the in the vitamin-mineral premix after 6 months was within ± 0.5 $\log_{10}$ CFU/g of the time zero count.

Stability in complete feed was investigated using three batches of the additive incorporated into a typical mash feed for pigs (maize, barley and soybean) and into a pelleted feed of the same composition (pelleting conditions 95°C for 30 s).[22] Samples of the mash and pelleted feed were placed in sealed foil bags and stored for up to 3 months at 25°C. Counts of total bacilli were made at the start and after 1 and 3 months. Essentially no reduction in total counts was seen in either the mash feed or the pelleted feed after pelleting. The effect of pelleting itself, obtained by comparison of the initial counts of the mash and pelleted feed, was small and less than 0.5 $\log_{10}$ difference.

A total of 10 subsamples were taken from the mash feed after 10 min mixing and analysed for total bacilli counts.[22] Based on the ten samples, the coefficient of variation was 1%, demonstrating homogeneous mixing.

In the absence of any significant reduction in counts in any of the above studies, it is considered unnecessary to monitor individual strains.

3.1.4. Conditions of use

The product is proposed for use in feed for pigs for fattening and minor porcine species at a minimum inclusion level of 1.5×10^8 CFU/kg complete feedingstuffs.

[redacted]

[16] Technical dossier/Section II/Annex II.1.4.1.1.
[17] Technical dossier/Section II/Annex II.1.4.1.2.
[18] Technical dossier/Section II/Annex II.1.5.2.
[19] Technical dossier/Section II/Annex II.1.5.3.
[20] Technical dossier/Section II/Annex II.4.1.1.
[21] Technical dossier/Section II/Annex II.4.1.2.
[22] Technical dossier/Section II/Annex II.4.1.3.

3.2. Safety

3.2.1. Safety for target animals, consumers and environment

The bacterial species *B. subtilis* and *B. amyloliquefaciens* are considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to safety assessment (EFSA, 2007; EFSA BIOHAZ Panel, 2017). This approach requires the identity of the strains to be conclusively established and evidence that the strains lack toxigenic potential and do not show acquired antimicrobial resistance determinants for antibiotics of human and veterinary importance.

In the view of the FEEDAP Panel, the identity of the three active agents is established and the lack of toxigenic potential confirmed. The two *B. amyloliquefaciens* strains do not show resistance to antibiotics of human and veterinary importance, and therefore, are presumed safe for the target species, consumers of products derived from animals fed the additive and the environment. The third strain, *B. subtilis* NRRL B-50510, showed a low level of resistance to streptomycin, for which acquired resistance genes were not identified. Therefore, it also complies with the QPS qualifications and is presumed safe for the target species, consumer and the environment. Since the other components of the additive do not give rise to concerns, Cinergy® Life B3 HiCon is also considered safe for the target species, consumer and the environment.

3.2.2. Safety for the user

No studies, other than those relating to dust formation, were provided. In the absence of data no conclusions on skin or eye irritancy or on dermal sensitisation can be made.

Owing to the proteinaceous nature of the active agents, the additive is considered a potential respiratory sensitiser. The particle size distribution and dusting potential make it unlikely that additive represents a respiratory hazard.

3.3. Efficacy

3.3.1. Efficacy for pigs for fattening

Four efficacy studies were performed, two in the same Member State and location and two in a non-EU country.

The first two trials were made at the same location using a common design and the same breed of pig (Table 1) but separated by a 3-month period. The 96 animals (equal numbers of male and female) in each trial were distributed according to body weight at the start to a total of 32 pens each containing three animals of the same sex. Sixteen pens (eight male and eight female pens) were allocated to each of the two treatments – a control group fed the basal diet and a test group given the basal diet supplemented with 1.5×10^8 CFU bacilli/kg feed. All feeds were analysed for the presence of bacilli, but results were confused by a high background count. Animals were fed mash diets containing maize, maize dry distillers grain with solubles (DDGs), wheat middlings and soybean meal in three phases. The duration of the trials was 96 days for trial 1[23] and 97 days for trial 2.[24] The parameters recorded were body weight and total pen feed intake at days 0, 42 and the end of the trial. From these data average daily weight gain, average daily feed intake and feed to gain ratio were calculated. At the end of the trials all pigs were slaughtered and carcasses evaluated. Data were analysed by analysis of variance (ANOVA) with the pen as the experimental unit. For performance data, the model used included dietary treatment, sex, block and the interaction between treatment and sex as main effects, while for carcass data the model included dietary treatment, sex and the interaction. Group means were compared using the Dunnett's test.

In the third trial, 120 pigs (Table 1) were distributed in a randomised block design across 30 pens, with 15 pens (8 of females and 7 of male barrows) per treatment with four pigs in each pen.[25] The two treatment groups were a control group given mash feeds in three phases based on maize, maize DDGs and soybean meal and a test group fed the same basal diets supplemented with the additive at 1.5×10^8 CFU/kg diet (confirmed for the test group). The duration of the trial was 111 days. Body weight measurement and feed consumption per pen were made every 3 weeks or on change of diet

[23] Technical dossier/Section IV/Annex IV.3.1.
[24] Technical dossier/Section IV/Annex IV.3.2.
[25] Technical dossier/Section IV/Annex IV.3.3.

and from these data average daily gain, average daily feed intake and feed to gain ratio calculated. Four male and four females per treatment were removed from the trial for a digestibility study (not reported) and the data arising from the pens from which pigs were removed (2, 3, 17 and 12) were excluded from the analysis of performance (in the last 2 phases). At the end of the trial 100 pigs were slaughtered and carcass composition assessed. Data were analysed by ANOVA with the pen as the experimental unit for performance measurements. For carcass composition data, the individual pig was used as the experimental unit. For all data, the model included treatment and sex as a fixed effect, and the interaction of treatment × sex.

The fourth trial involved 56 pigs (Table 1) which were distributed in a randomised block design across 14 pens with seven pens per treatment with four pigs (2 female and 2 male barrows) in each pen.[26] Pigs were introduced into the trial in three batches at weekly intervals to ensure approximately equal start weights. The two treatment groups were a control group given mash feeds in three phases based on maize, maize DDGs and soybean meal and a test group fed the same basal diets supplemented with the additive at 1.5×10^8 CFU/kg diet (confirmed for the test group). The overall duration of the trial was 109 days but, because of the different start dates, the average duration for individual animals ranged between 84 and 92 days for the control group and 84 and 93 days for the treatment group. It is presumed that individual animals were retained on trial until reaching a body weight of approximately 120 kg. Body weight and feed consumption was recorded at the start, at each dietary interval and at the end of the trial and from these data average daily gain, average daily feed intake and feed to gain ratio calculated. The data were analysed by ANOVA with the pen as the experimental unit. Initial bodyweight was included as a covariate. Group means were compared using the Tukey's test.

Table 1: Details on the study design for the studies performed in pigs for fattening

Study (duration in days)	Breed	Total animals replicates/ treatment × animals/ replicate	Intended concentration in feed (CFU/kg feed)	Analysed concentration in feed (CFU/kg feed)	Basal diets (main ingredients) form
1 (96)	Pietrain × (Landrace × Yorkshire)	96 16 × 3	0 1.5×10^8	$180/2.1/6.4 \times 10^6$ $0.7/0.4/1.4 \times 10^8$	(maize, maize DDGs, wheat middlings and soybean) mash
2 (97)	Pietrain × (Landrace × Yorkshire)	96 16 × 3	0 1.5×10^8	$4.0/160/4.0 \times 10^6$ $0.6/0.3/0.6 \times 10^8$	(maize, maize DDGs, wheat middlings and soybean) mash
3 (111)	PIC Genetiporc 6.0 × Genetiporc F25	120 15 × 4	0 1.5×10^8	$4.5/8.0/1.3 \times 10^6$ $1.2/1.4/1.0 \times 10^8$	(maize, maize DDGs and soybean meal) mash
4 (109)	(Yorkshire × Landrace) × Duroc; Yorkshire × Duroc	56 7 × 4	0 1.5×10^8	$6.3/1.2/1.0 \times 10^6$ $0.2/2.0/0.3 \times 10^8$	(maize, maize DDGs and soybean meal) mash

CFU: colony forming unit.

Table 2: Effects of the additive Cinergy® Life B3 HiCon on the performance of pigs for fattening

Trial no	Additive (CFU/kg feed)	Initial body weight (kg)	Feed intake (kg/day)	Final body weight (kg)	Daily weight gain (g/day)	Feed to gain ratio
1	0	29.9	2.01	107.5	808.2	2.49[a]
	1.5×10^8	29.9	1.96	108.0	813.7	2.41[b]
2	0	31.6	1.91	105.5	761.8	2.52[a]
	1.5×10^8	31.5	1.89	107.8	778.6	2.43[b]
3	0	23.5	2.00	129.1	942.0	2.13
	1.5×10^8	23.1	1.98	128.9	938.0	2.11

[26] Technical dossier/Section IV/Annex IV.3.4 and Supplementary information July 18/Annex II.

Trial no	Additive (CFU/kg feed)	Initial body weight (kg)	Feed intake (kg/day)	Final body weight (kg)	Daily weight gain (g/day)	Feed to gain ratio
4	0	28.9	2.95[a]	122.2	1030.0	2.86
	1.5×10^8	28.5	2.75[b]	121.9	1030.0	2.68

CFU: colony forming unit.
[a,b]: Means within a column and study with different superscript letters are significantly different at $p < 0.05$.

The Panel notes the abnormal high *Bacilli* counts in one control diet sample in studies 1 and 2. The applicant justified these values by a high background contamination and subjected all feed samples to confirmation of the presence of the active agents. All control feed samples proved negative and the treated feeds proved positive. Positive refers to a sample in which the majority of the colonies have a morphology similar to that of the three added Cinergy Life B3 HiCon *Bacillus* strains. The FEEDAP Panel does not consider this to be of concern.

No mortalities occurred in either of the first two trials, but in trial 2 two animals from the Cinergy group had to be withdrawn because of ill-health. There were no significant differences in growth or feed intake in either trial but, in both cases, there was a significant improvement in feed to gain ratio (Table 2). No significant effects were seen on carcass composition in trial 1 (liveweight, hot carcass, killing out percentage, % offals, % lean meat). In trial 2, the backfat depth was significantly reduced (15.1 mm vs. 16.7 mm) and lean meat percentage significantly increased (61.5% vs. 59.9%) in the treated group compared to control values.

In the third study, two pigs died during the trial and two were removed (one from each treatment group in both cases) because of enteric disease. Overall, there were no significant differences between the control and treated groups in performance (Table 2) or carcass composition (not reported).

In the last study, no mortalities occurred and no animals were removed from the trial. Feed intake was significantly lower in the treated group compared to the control animals without an effect on final body weight (Table 2). This led to an improved feed to gain ratio, although the difference did not reach significance.

Since significant effects were observed in two studies only, the applicant performed an analysis pooling the data shown in Table 2, including the effect of the treatment, study and their interaction. No interactions were found between treatment and study, and a significant difference was found for feed to gain ratio between the treatments (control 2.50 vs. additive 2.43, $p = 0.019$).[27]

Conclusions on efficacy

Two of the four individual studies showed a significant improvement in feed to gain ratio in favour of the additive. Pooling of the data from the four studies showed a potential of the additive at a minimum inclusion level of 1.5×10^8 CFU/kg complete feed to improve the feed to gain ratio of pigs for fattening.

3.3.2. Efficacy for minor porcine species

Since a potential to improve the feed to gain ratio in pigs for fattening given the additive at the recommended inclusion level of 1.5×10^8 CFU/kg complete feed was shown, the same conclusion can be extrapolated to minor porcine species at the same minimum application rate and for an equivalent growth phase.

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[28] and Good Manufacturing Practice.

4. Conclusions

The three active agents comprising the additive (*B. amyloliquefaciens* NRRL B-50508 and B-50509 and *B. subtilis* NRRL B-50510) meet the requirements of the QPS approach to safety assessment and

[27] Technical dossier/Section IV/Annex IV.3.5.
[28] 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.

are 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 give rise to concerns, Cinergy® Life B3 HiCon is also considered safe for the target species, consumer and the environment.

In the absence of data, no conclusions on skin or eye irritancy or on the potential for dermal sensitisation can be made. Owing to the proteinaceous nature of the active agents, the additive is considered a potential respiratory sensitiser. However, the low dusting potential makes it unlikely that additive poses a risk for the respiratory system.

Cinergy® Life B3 HiCon showed a potential to improve the feed to gain ratio in pigs for fattening at the minimum inclusion level of 1.5×10^8 CFU/kg complete feed. This conclusion is extrapolated to minor porcine species at the same application rate and for an equivalent growth phase.

Documentation provided to EFSA

1) Cinergy® Life B3 HiCon. *Bacillus amyloliquefaciens* AGTP BS918 (NRRL B-50508), *Bacillus amyloliquefaciens* AGTP BS1013 (NRRL B-50509), *Bacillus subtilis* AGTP BS3BP5 (NRRL B-50510). Zootechnical additive for pigs for fattening and minor porcine species functional group: gut flora stabiliser. October 2017. Submitted by Cargill Incorporated, represented By Provimi Holding BV.
2) Cinergy® Life B3 HiCon. *Bacillus amyloliquefaciens* AGTP BS918 (NRRL B-50508), *Bacillus amyloliquefaciens* AGTP BS1013 (NRRL B-50509), *Bacillus subtilis* AGTP BS3BP5 (NRRL B-50510). Zootechnical additive for pigs for fattening and minor porcine species functional group: gut flora stabiliser. Supplementary information. September 2018. Submitted by Cargill Incorporated, represented By Provimi Holding BV.
3) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Cinergy® Life B3 HiCon.
4) Comments from Member States.

Chronology

Date	Event
30/10/2017	Dossier received by EFSA
16/11/2017	Reception mandate from the European Commission
27/2/2019	Application validated by EFSA – Start of the scientific assessment
3/4/2018	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
8/4/2018	Comments received from Member States
31/5/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation and efficacy*
16/7/2018	Reception of supplementary information from the applicant – Scientific assessment re-started
12/9/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended *Issues: characterisation*
7/12/2018	Reception of supplementary information from the applicant – Scientific assessment re-started
27/2/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

ANOVA	analysis of variance
BW	body weight
CFU	colony-forming unit
CG	chemical group
CV	coefficient of variation
DDGs	dry distillers grain with solubles
EURL	European Union Reference Laboratory
LOQ	limit of quantification
MIC	minimum inhibitory concentration
PCB	polychlorinated biphenyl
PCDD/F	polychlorinated dibenzo-*p*-dioxins and dibenzofurans
PCR	polymerase chain-reaction
QPS	qualified presumption of safety
RAPD	randomly amplified polymorphic DNA
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 Cinergy® Life B3 HiCon

Cinergy® Life B3 HiCon is the trade name of a preparation based on viable spores of the three non-genetically modified strains *Bacillus amyloliquefaciens* AGTP BS918 (NRRL B-50508), *Bacillus amyloliquefaciens* AGTP BS1013 (NRRL B-50509) and *Bacillus subtilis* AGTP BS3BP5 (NRRL B-50510).

In the current application, authorisation is sought under Article 4(1) for this product 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 pigs for fattening and minor porcine species.

The *feed additive* is to be marketed as a powder containing a minimum content of total active substances of 2.5×10^9 Colony Forming Unit (CFU)/g. The *feed additive* is intended to be used directly in *feedingstuffs* or through *premixtures* at a minimum dose of 1.5×10^8 CFU/kg of complete *feedingstuffs.*

For the identification of *Bacillus amyloliquefaciens* AGTP BS918, *Bacillus amyloliquefaciens* AGTP BS1013 and *Bacillus subtilis* AGTP BS3BP5, the EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised methodology for genetic identification of bacterial strains.

For the enumeration of all *Bacillus* spp. strains (AGTP BS918, AGTP BS1013 and AGTP BS3BP5) in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted the ring-trial validated spread plate CEN method EN 15784. Based on the performance characteristics available, 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.