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Safety and efficacy of a feed additive consisting on *Ligilactobacillus animalis* ATCC PTA-6750 (formerly *Lactobacillus animalis*) for all animal species (Chr. Hansen A/S)

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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 additive consisting of *Ligilactobacillus animalis* (formerly known as *Lactobacillus animalis*) ATCC PTA-6750 when used as a technological additive (acidity regulator, preservative and hygiene condition enhancer) in feed and water for drinking for all animal species. The product is intended for use as a single strain at a minimum inclusion level of 5×10^6 CFU/L or CFU/kg in water, liquid and dry feeds. No minimum inclusion level is proposed by the applicant when used in combination with other microbial technological additives. The bacterial species *L. animalis* is considered by EFSA to be eligible for the qualified presumption of safety approach. As the identity of the strain has been clearly established and it did not show acquired resistance to antibiotics of human and veterinary importance, the use of the strain in animal nutrition is considered safe for the target species, consumers and the environment. No conclusions can be drawn on the skin/eye irritancy or skin sensitisation potential of the product, but it should be considered a respiratory sensitiser. Exposure of users by inhalation is likely. The Panel is not in the position to conclude on the efficacy of *L. animalis* ATCC PTA-6750 when used in animal nutrition as an acidity regulator, preservative or hygiene condition enhancer due to lack of data. The studies provided showed that *L. animalis* ATCC PTA-6750 when used in combination with *Propionibacterium freudenreichii* ssp. *shermanii* ATCC PTA-6752 has the potential to act as an acidity regulator. However, the Panel has reservations on the effects of this mixture in practical use conditions.

Keywords: technological additive, acidity regulator, preservative, hygiene condition enhancer, *Ligilactobacillus animalis* ATCC PTA-6750, safety, efficacy

Requestor: European Commission

Question number: EFSA-Q-2016-00713

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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 Chr. Hansen A/S[2] for authorisation of the product consisting of *Ligilactobacillus animalis* ATCC PTA-6750 (formerly *Lactobacillus animalis*) when used as a feed additive for all animal species (category: technological additives; functional groups: acidity regulators, preservatives and hygiene condition enhancers).

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). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 23 December 2016.

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 consisting of *L. animalis* ATCC PTA-6750, when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

The product under assessment is a preparation containing viable cells of *L. animalis* ATCC PTA-6750. It has not been previously authorised as a feed additive 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[3] in support of the authorisation request for the use of the product consisting of *L. animalis* ATCC PTA-6750, as a feed additive.[4]

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 the product consisting of *L. animalis* ATCC PTA-6750 is in line with the principles laid down in Regulation (EC) No 429/2008[4] and the relevant guidance documents: Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011), Guidance on technological additives (EFSA FEEDAP Panel, 2012a), Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA FEEDAP Panel, 2012b) and Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018).

[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] Chr. Hansen A/S, 10-12 Boege Allé, 2970 – Hoersholm, Denmark.
[3] FEED dossier reference: FAD-2016-0068.
[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/sites/jrcsh/files/finrep-fad-2016-0068-lacto_animalis.pdf

3. Assessment

The product under assessment is based on a preparation of viable cells of a single strain *L. animalis* (formerly known as *Lactobacillus animalis*, Zheng et al., 2020) intended to be used as a technological additive (functional groups: preservatives, acidity regulators and hygiene condition enhancers) in feed and water for drinking for all animal species. It will be hereafter referred to as LA51.

3.1. Characterisation

3.1.1. Characterisation of the active agent

The strain was originally isolated from a calf ileum and is deposited in the American Type Culture Collection (ATCC) with the accession number PTA-6750.[6] It has not been genetically modified.

The strain ATCC PTA-6750 was identified at species level as *L. animalis* by whole genome sequence (WGS) analysis.[7] The digital DNA–DNA hybridisation (dDDH) value was 94.6% compared with the genome sequence of the type strain *L. animalis* DSM 20602^{T}.[8] The 16S rRNA gene sequence (1,489 bp) was also compared with the database LTPs132_SSU.arb obtaining 99.9% sequence identity with the *L. animalis* type strain.

The strain ATCC PTA-6750 has the capability for fermenting ribose and producing both lactic and acetic acids, fulfilling the requirements for a facultative heterofermentative organism.[9] Therefore, the susceptibility of the bacterial strain was tested against the list of antibiotics recommended by EFSA (EFSA FEEDAP Panel, 2012b) for facultative heterofermentative lactobacilli. All minimum inhibitory concentration (MIC) values for the strain were equal to or below the corresponding cut-off values, except for chloramphenicol and kanamycin. The MIC values for chloramphenicol and kanamycin were 8 and 256 mg/L, respectively. These values are, respectively, one and two dilutions above the cut-off values.[10] Exceeding the cut-off value by one dilution is considered to be within the normal range of variation, and thus, not a matter of concern.

The WGS of the strain was interrogated [redacted] for the presence of antimicrobial resistance (AMR) genes [redacted][11] No relevant hits were identified. Although the strain was resistant to kanamycin, since no acquired AMR genes were found in the WGS, it can be assumed that this resistance does not raise safety concerns.

3.1.2. Characterisation of the product

[redacted] The final additive is a powder [redacted] with [redacted] a minimum guaranteed concentration of 2×10^{11} colony forming units (CFU) per gram of additive.[12]

Analysis of six batches of the standardised product showed a mean value of 3.1×10^{11} CFU/g (range $2.4–4.1 \times 10^{11}$ CFU/g).[13]

Specifications are set for total coliforms (< 10 CFU/g), *Salmonella* spp. (no detection in 25 g), *Escherichia coli*, (< 1 CFU/g) and total yeasts and filamentous fungi (< 10 CFU/g). Analyses of six batches of the standardised product[14] and three additional batches of the cell concentrate prior to standardisation[13] confirmed compliance with these specifications.[15] Other three batches of the standardised product were examined for the presence of heavy metals (Hg, Cd and Pb), arsenic and aflatoxins B1, B2, G1 and G2.[16] In all cases, heavy metals and arsenic were found only in trace amounts

[6] Technical dossier/Section II/Annex II.2.1.2.1 and Annex II.2.1.2.2.
[7] Technical dossier/Supplementary information November 2020/Annex Q1.
[8] Alias *Ligilactobacillus animalis* NBRC 15882^{T}.
[9] Technical dossier/Section II/Annex 2.1.2.4. and Supplementary information November 2020/Annex Q2.
[10] Technical dossier/Section II/Annex II.2.2.2.1.; Annex II.2.2.2.2. and Annex II.2.2.2.3.
[11] Technical dossier/Supplementary information November 2020/Annex Q3.
[12] Technical dossier/Section II/Annex II.3.1.1.; Annex II.3.2.1. and Annex II.3.2.2.
[13] Technical dossier/Section II/Annex II.1.3.
[14] Technical dossier/Section II/Annex II.1.4.1.
[15] Technical dossier/Section II/Annex II.1.4.2.
[16] Technical dossier/Section II/Annex II.1.4.3.

(Hg < 0.01 mg/kg, Cd < 0.05–0.07 mg/kg, As: 0.07–0.08 mg/kg and Pb < 0.05–0.18 mg/kg) which do not give rise to safety concerns.[17] No aflatoxins were detected in any of the tested samples.[18]

The average bulk density is 600 kg/m^3 (range: 580–720 kg/m^3). The dusting potential of three batches of the standardised product measured with the Stauber–Heubach dustmeter showed an average value of 19.9 g/m^3 (range: 18.8–20.6 g/m^3). The particle size distribution of the same three batches was determined using laser diffraction. Results showed that on average 42% (v/v) of the additive consist of particles with diameter below 100 μm, 28% below 50 μm and 17% below 10 μm.[19]

3.1.3. Stability and homogeneity

The applicant declared a minimum shelf-life of 12 months when stored in the original container at –30°C or lower. Stability was assessed in the cell concentrate prior to standardisation at –30°C for 12, 14 or 18 months (one batch, one replicate each). Losses of the active agent counts were < 0.5 Log in all cases.[20] The Panel notes that the storage conditions proposed to guarantee the stability of LA51 (at least –30°C) are not compatible with standard farming conditions.

The applicant declares that LA51 should be incorporated to feed shortly before intended consumption. A short-term stability study was conducted to monitor viability of LA51 when mixed with liquid feeds (liquid pig feed, calf milk replacer and isotonic glucose-saline solution) and water at a target concentration of 1×10^{11} CFU/L. The samples were incubated in aerobiosis at 25°C for 24 h (timepoints 2, 4, 6, 8 and 24 h). Losses in lactic acid bacteria counts remained < 0.5 Log at all timepoints in liquid pig feed and calf milk replacer, only during the first 8 h in water and during the first 6 h in the isotonic glucose-saline solution.[21] However, the Panel notes that (i) the concentration tested was several orders of magnitude higher than the proposed inclusion level in feeds and (ii) the conditions of the test would not mimic the standard feed preparation (i.e. sterilisation of matrices). No data on dry feed were provided.

To investigate if the additive (one batch) can be homogeneously distributed into water and the same liquid feeds described above at an intended concentration of 1×10^{11} CFU/L, 10 sub-samples were collected and subjected to lactic acid bacteria enumeration.[22] Results showed a coefficient of variation < 1% in all samples. No data on dry feed were supplied.

3.1.4. Conditions of use

L. animalis ATCC PTA-6750 is intended to be used in solid and liquid feeds and in water for drinking for all animal species at a minimum inclusion level of 5×10^6 CFU/kg or CFU/L. The applicant states that this inclusion level regards its individual use. In case the additive is used in combination with other microorganisms, no minimum recommended levels are proposed. It is to be incorporated to feed shortly before intended consumption.[23]

3.2. Safety

3.2.1. Safety for the target species, consumers and environment

The species *L. animalis* is considered by EFSA to be eligible for the Qualified Presumption of Safety (QPS) approach (EFSA, 2007; EFSA BIOHAZ Panel, 2020). This approach requires the identity of the strain to be conclusively established and evidence that the strain does not show acquired resistance to antibiotics of human and veterinary importance. In the view of the FEEDAP Panel, the identity of the strain has been established as *L. animalis* and the antibiotic resistance qualification met. Consequently, *L. animalis* ATCC PTA-6750 is considered to meet the criteria to be considered as presumably safe for the target species, consumers and the environment.

[17] Limits of detection (LOD) for lead: 0.02 mg/kg and mercury: 0.010 mg/kg. Those for cadmium and arsenic were not specified.
[18] LOD: 0.5 μg/kg.
[19] Technical dossier/Section II/Annexes II.1.5.1,II. 1.5.2., II.1.5.3., II.1.5.4. and II.1.5.5.
[20] Technical dossier/Section II/Annex II.4.1.1.
[21] Technical dossier/Section II/Annex II.4.1.3.
[22] Technical dossier/Section II/Annex II.4.2.
[23] Technical dossier/Spontaneous submission July 2017/Annex 5_LA51_AddI_14_7_2017.

3.2.2. Safety for the user

No specific data on skin/eye irritation or skin sensitisation were provided for the additive under application. Therefore, no conclusions can be drawn on the skin/eye irritancy or skin sensitisation potential of LA51. The high dusting potential (19.9 g/m^3) suggests that exposure by inhalation is possible. Given the proteinaceous nature of the active agent, LA51 should be considered a respiratory sensitiser.

3.3. Efficacy

The applicant has produced a total of sixteen studies to support the efficacy of the LA51 as preservative and hygiene condition enhancer (one *in vitro* study and two *in vivo* studies) and as acidity regulator (13 *in vitro* studies).

To prove the effects as a preservative and hygiene condition enhancer, an *in vitro* study was conducted where the product was grown in sterilised nutrient broth under anaerobic conditions at 37°C.[24] However, this study was disregarded as it does not reflect practical farming conditions. The two *in vivo* studies provided[25] were not designed to support LA51 effects on feed, but on the carriage of several gastrointestinal pathogens in the animals' gut (i.e. beef calves and chickens for fattening). Since the relevant parameters in feed were not measured, no conclusions can be drawn from these studies.

Therefore, no suitable data to support the use of the additive as preservative and hygiene condition enhancer were available.

To support the efficacy as acidity regulator, thirteen *in vitro* studies have been submitted. However, six of them were not further considered because none of the matrices used can be deemed as representative of feedstuffs in which LA51 is intended to be used (two different pig feeds, three different calf milk replacers and a glucose-saline solution).[26] The pig feeds and calf milk replacers were diluted with a growth medium (nutrient broth) used to represent gut chime. These solutions and the incubation conditions (i.e. sterilisation of the matrices, anaerobiosis, high temperature (37°C)) are not considered to represent normal feedingstuffs, nor feeding conditions. In an additional *in vitro* study to support the efficacy of LA51 as acidity regulator, the matrices considered were valid representatives of feedstuffs, water and pig feed diluted with water.[27] However, the sterilisation of the matrices before the addition of LA51 and the artificial incubation conditions (i.e., anaerobiosis, high temperature (37°C)) do not reflect practical use conditions, raising uncertainty about the relevance of the results. Therefore, also this study was not further considered.

The remaining six *in vitro* studies were aimed at supporting the effects of LA51 as acidity regulator. In study 1,[28] the additive LA51 was prepared at a concentration of 6.5×10^9 CFU/kg with lactose before being used in the feed (calf milk replacer, representing liquid feeds for young ruminants). In study 2,[29] LA51 was prepared at a concentration of 8×10^8 CFU/kg with lactose and mixed with water in a 40:60 ratio previously to be added to the feed (high fibre dry mix, raw fibre content 16%). In the remaining four of these studies (studies 3–6) LA51 was used in combination with another additive (*Propionibacterium freudenreichii* ssp. *shermanii* PF24, Table 1). In order to inoculate feeds with the additives in studies 3–6, a premix containing both additives at a concentration of $6.3–8.4 \times 10^{13}$ total CFU/kg with lactose (39.5%) and silicon dioxide (1%) was prepared. In study 3,[30] this premix and the control (prepared with equivalent amounts of lactose) were mixed with water in a 60:40 ratio to simulate water uptake during transport. In studies 4,[31] 5[32] and 6[33] this premix was further diluted with lactose before being used in the treated feeds (study 4: commercial milk for cats, study 5: complete feed (25% wheat, 25% barley, 25% maize, 25% soybean), representing dry feeds for pigs, ruminants and poultry and study 6: high fibre dry feed mix, representing total mixed ration for ruminants). In all these studies, the control feeds were prepared with equivalent amounts of lactose.

In all studies, at time zero and different subsequent timepoints, samples from six replicates were taken for measuring the pH, except in study 4 in which only one replicate was analysed at time zero

[24] Technical dossier/Section IV/Annex IV.1.1.
[25] Technical dossier/Supplementary information May 2019/Annexes IV.1.13. and IV.1.14.
[26] Technical dossier/Spontaneous submission_180717/Annexes IV.1.3., IV.1.4., IV.1.5. and IV.1.6.
[27] Technical dossier/Section IV/Annex IV.1.2.
[28] Technical dossier/Supplementary information May 2019/Annex IV.1.11.
[29] Technical dossier/Supplementary information May 2019/Annex IV.1.12.
[30] Technical dossier/Supplementary information May 2019/Applicant Reply and Annex IV.1.9.
[31] Technical dossier/Supplementary information May 2019/Applicant Reply and Annex IV.1.7.
[32] Technical dossier/Supplementary information May 2019/Applicant Reply and Annex IV.1.8.
[33] Technical dossier/Supplementary information May 2019/Applicant Reply and Annex IV.1.10.

and in study 2 in which only three replicates were taken. In studies 2, 3, 5 and 6 the samples were diluted with water (ratio 1:2 in studies 3, 5 and 6; ratio 1:9 in study 2) to measure the pH. The data were analysed with an analysis of variance (ANOVA) with the replicate as experimental unit. Differences were considered significant at a level of at least $p < 0.05$. The details of the experimental design and results are shown in Table 1.

Table 1: Summary of the *in vitro* trials of different feed matrices inoculated with LA51 alone and in combination with PF24

Study	Matrices	N° replicates aerobic incubation conditions	Treatment LA51/PF24 (CFU/L or kg)	pH at the timepoints					
				0 h	6 h	8 h	24 h	48 h	72 h
1	Calf milk replacer	6	Control	6.62	–	–	4.90[a]	–	–
		24 h, 25 °C	1.1×10^9/–	6.64	–	–	4.67[b]	–	–
2	High fibre dry mix	3	Control	6.22[a]	6.06[a]	–	6.31[a]	–	–
		24 h, 25 °C	8.0×10^8/–	6.41[b]	6.30[b]	–	6.08[b]	–	–
3	Lactose-based complementary feed	6	Control	6.64	–	5.48[a]	5.30[a]	–	5.23[a]
		72 h, 30°C	3.9×10^{13}/ 7.3×10^{13}	6.65	–	5.35[b]	5.13[b]	–	4.98[b]
4	Commercial milk for cats	6	Control	6.47	–	6.48[a]	6.42[a]	–	–
		24 h, 35°C	3.5×10^7/ 7.1×10^7	6.49	–	6.45[b]	6.25[b]	–	–
5	Complete feed	6	Control	6.21	–	6.28[a]	6.58[a]	–	–
		24 h, 35°C	3.5×10^{11}/ 7.1×10^{11}	6.18	–	5.38[b]	4.71[b]	–	–
6	High fibre dry mix	6	Control	5.65	–	–	5.60	5.50[a]	–
		48 h, 30 °C	3.5×10^7/ 7.1×10^7	5.47	–	–	5.59	4.75[b]	–

CFU: colony forming unit.
LA51: *L. animalis* ATCC PTA-6750.
PF24: *Propionibacterium freudenreichii* ssp. *shermanii* ATTC PTA-6752.
a, b: Means in the same column within study are significantly different compared to control with $p \leq 0.05$.
–: not measured.

In the only two studies where LA51 was applied alone (studies 1 and 2), a significantly lower pH was observed in the treated samples compared with controls. Nevertheless, in the absence of a third study showing positive effect, there is insufficient evidence to conclude on the efficacy of LA51 as acidity regulator.

The four studies, where LA51 was used in combination with another additive (studies 3–6), showed a significant reduction of pH for all treated samples compared to the controls, starting from 8 h after preparation.

Considering the conditions in which the effects were observed (e.g. high incubation temperature (35°C), composition of the feeds chosen to represent liquid feedingstuffs for all animal species) and the conditions of use proposed (to be stored at –30°C and added just before consumption), the FEEDAP Panel has reservations on the effects of the simultaneous use of LA51 and PF24 in practical use conditions.

The Panel notes that the proposed conditions of use of the additive (to be stored at –30°C and added just before consumption) may limit the relevance of the results seen in the efficacy studies under practical farm conditions. This would apply to the additive both when used alone and in combination with PF24.

3.3.1. Conclusions on efficacy

The Panel is not in the position to conclude on the efficacy of LA51 when used alone as an acidity regulator in feed or water. The studies provided showed that LA51 when used in combination with *P. freudenreichii* ssp. *shermanii* ATTC PTA-6752 (PF24) has the potential to act as an acidity regulator in feeds. However, the Panel has reservations on the effects of this mixture in practical use conditions.

In the absence of valid studies investigating relevant endpoints to cover the efficacy of LA51 as preservative or hygiene condition enhancer (e.g. potential human or animal enteropathogens or undesirable bacteria), no conclusion can be drawn on the efficacy in relation to these two functional groups.

4. Conclusions

Based on the QPS approach to safety assessment, *L. animalis* ATCC PTA-6750 is presumed safe for the target species, consumers and the environment.

Due to the absence of data, no conclusions can be drawn on the skin/eye irritancy or skin sensitisation potential of the product under assessment. Given the proteinaceous nature of the active agent, *L. animalis* ATCC PTA-6750 should be considered a respiratory sensitiser. Exposure of users by inhalation is likely.

The Panel is not in the position to conclude on the efficacy of *L. animalis* ATCC PTA-6750 when used alone as an acidity regulator in feed or water. The studies provided showed that *L. animalis* ATCC PTA-6750 when used in combination with *P. freudenreichii* ssp. *shermanii* ATTC PTA-6752 has the potential to act as an acidity regulator in feeds. However, the Panel has reservations on the effects of this mixture in normal practical use conditions.

In the absence of valid studies investigating relevant and specific endpoints, no conclusion can be drawn on the efficacy of *L. animalis* ATCC PTA-6750 as preservative or hygiene condition enhancer.

5. Documentation as provided to EFSA/Chronology

Date	Event
26/10/2016	Dossier received by EFSA. *Lactobacillus animalis* LA51 (ATCC PTA-6750) for all animal species. Submitted by Chr. Hansen A/S.
11/11/2016	Reception mandate from the European Commission
23/12/2016	Application validated by EFSA – Start of the scientific assessment
23/03/2017	Comments received from Member States
20/04/2017	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
18/07/2017	Reception of spontaneous supplementary information from the applicant
12/01/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: efficacy*
07/05/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
04/09/2020	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation of the strain*
04/11/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
10/02/2021	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Hilbert F, Lindqvist R, Nauta M, Peixe L, Ru G, Simmons M, Skandamis P, Suffredini E, Cocconcelli PS, Fernández Escámez PS, Maradona MP, Querol A, Suarez JE, Sundh I, Vlak J, Barizzone F, Correia S and Herman L, 2020. Scientific Opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA (2017–2019). EFSA Journal 2020;18(2):5966, 56 pp. https://doi.org/10.2903/j.efsa.2020.5966

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Abbreviations

AMR	antimicrobial resistance
ANOVA	analysis of variance
EFSA BIOHAZ Panel	EFSA Panel on Biological Hazards
CFU	colony forming unit
dDDH	digital DNA–DNA hybridisation
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
LOD	limit of detection
MIC	minimum inhibitory concentration
PFGE	pulsed field gel electrophoresis
QPS	Qualified Presumption of Safety
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 *Lactobacillus animalis* LA51 (ATCC PTA-6750)

In the current application authorisation is sought under Article 4(1) for *Lactobacillus animalis*[34] *LA51 (ATCC PTA-6750)* under the category 1 'technological additives' and the functional groups (a) 'preservatives', (j) 'acidity regulators' and (n) 'hygiene condition enhancers', 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* contains as active substance viable cells of the non-genetically modified strain *Lactobacillus animalis LA51 (ATCC PTA-6750)*. The *feed additive* is to be marketed as a powder containing a minimum *Lactobacillus animalis LA51 (ATCC PTA-6750)* content of 2 × 1011 Colony Forming Unit (CFU)/g. The *feed additive* is intended to be used in *water* and liquid *feedingstuffs* at a minimum dose of 1 × 1011 CFU/l, and in dry *feedingstuffs* at a minimum dose of 2 × 1011 CFU/kg.

For the identification of *Lactobacillus animalis LA51 (ATCC PTA-6750)*, 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 *Lactobacillus animalis LA51 (ATCC PTA-6750)* in *feed additive*, *feedingstuffs* and *water,* the Applicant submitted the ring-trial validated spread plate method EN 15787. 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.

[34] Now designated as *Ligilactobacillus animalis*.