SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 3 July 2019

doi: 10.2903/j.efsa.2019.5787

Safety and efficacy of *Bacillus licheniformis* DSM 32457 as a silage additive for all animal 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 *Bacillus licheniformis* DSM 32457 as a silage additive for all animal species. *Bacillus licheniformis* DSM 32457 is presumed safe for the target species, consumers of products from animals fed treated silage and the environment. In the absence of data, the FEEDAP Panel cannot conclude on the potential of the additive to be a skin/eye irritant or skin sensitiser. Given the proteinaceous nature of the active agent, the additive should be considered a potential respiratory sensitiser. In the absence of appropriate data, the FEEDAP Panel cannot conclude on the efficacy of *Bacillus licheniformis* DSM 32457 as a silage additive.

Keywords: *Bacillus licheniformis*, technological additives, silage additive, safety, efficacy, QPS

Requestor: European Commission

Question number: EFSA-Q-2018-00690

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Acknowledgements: The Panel wishes to thank the following for the support provided to this scientific output: Lucilla Gregoretti and Rosella Brozzi.

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Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Kouba M, Kos Durjava M, López-Alonso M, López Puente S, Marcon F, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Glandorf B, Herman L, Prieto Maradona M, Saarela M, Anguita M, Galobart J, Holczknecht O, Manini P, Pizzo F, Tárres-Call J, Pettenati E and Mayo B, 2019. Scientific Opinion on the safety and efficacy of *Bacillus licheniformis* DSM 32457 as a silage additive for all animal species. EFSA Journal 2019;17(8):5787, 9 pp. https://doi.org/10.2903/j.efsa.2019.5787

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

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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 EnviroSystems (UK) Ltd[2] for authorisation of the product *Bacillus licheniformis* DSM 32457 (Optimize), when used as a feed additive for all animal species (category: technological additives; functional group: silage additives).

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 7 December 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 *Bacillus licheniformis* DSM 32457 (Optimize), when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

The additive is a preparation containing viable cells of *Bacillus licheniformis* DSM 32457. 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 *Bacillus licheniformis* DSM 32457 as a feed additive.

European Food Safety Authority (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 Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) to assess the safety and the efficacy of *Bacillus licheniformis* DSM 32457 is in line with the principles laid down in Regulation (EC) No 429/2008[5] and the relevant guidance documents: Guidance on technological additives (EFSA FEEDAP Panel, 2012a), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b) and Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c).

[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] EnviroSystems (UK) Ltd, Barton Cross Park, Barton Lane, PR3 5AX, Preston, United Kingdom.
[3] FEED dossier reference: FAD-2018-0064.
[4] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2018-0064-optimize.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 of spores of *Bacillus licheniformis* DSM 32457[6] intended for use as a technological additive (silage additive) for all animal species.

3.1. Characterisation

3.1.1. Characterisation of the active agent

The *Bacillus licheniformis* strain [redacted] It is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen with the accession number DSM 32457.[8] It has not been genetically modified. Taxonomical identification of the strain as *B. licheniformis* was established [redacted]

Bacillus licheniformis DSM 32457 was tested for antibiotic susceptibility using broth microdilution techniques. [redacted] it is not considered to be a hazard.

The toxigenic potential of *B. licheniformis* DSM 32457 was investigated in a cytotoxicity assay with Vero cells [redacted] according to Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018).[10] [redacted] No cytotoxic effects were detected.

3.1.2. Characterisation of the product

[redacted] The resulting product, *B. licheniformis* active powder is standardised to [redacted] colony-forming units (CFU)/g. The *B. licheniformis* active powder [redacted] is then blended with [redacted] to obtain the product under assessment with a minimum specified content of [redacted] CFU/g.

Analysis of five batches of the final product showed a mean value of [redacted][11] The same five batches were analysed for microbial contamination.[11]

[6] In-house number of the strain: ENV01.
[redacted]
[8] Technical dossier/Section II/Annex_II_2.2.1.1.
[redacted]
[10] Technical dossier/Section II/Annex_II_2.2.2.1.
[11] Technical dossier/Section II/Annex_II_2.1.3.

Enterobacteriaceae, *Escherichia coli*, yeasts and filamentous fungi and *Bacillus cereus* were not detected.[12] *Salmonella* was absent in 25 g.[13] Aflatoxins and heavy metals were tested in three batches.[14] [redacted]

Three batches of the additive were examined for dusting potential (Stauber–Heubach method) and for particle size distribution by laser diffraction.[17] The dusting potential ranged between 1.75 and 2.41 g/m^3. In average, 71.4% (v/v) of the additive consists of particles below 100 μm, 43.8% below 50 μm and 5.0% below 10 μm.

3.1.3. Stability

[redacted]

3.1.4. Conditions of use

The additive is intended for use with easy to ensile forages for all animal species at a proposed minimum concentration of 5×10^7 CFU/kg forage and a maximum concentration of 1.2×10^8 CFU/kg forage.[7] The applicant recommends applying it as an aqueous suspension.

3.2. Safety

3.2.1. Safety for the target species, consumers and environment

The species *B. licheniformis* is 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 strain to be conclusively established and evidence that the strain does not show acquired resistance to antibiotics of human and veterinary importance and lack of toxigenic potential.

The FEEDAP Panel noted that the identity of the strain was established as *B. licheniformis*. Evidence was provided on the lack of toxigenic potential of the strain and on the absence of acquired antimicrobial resistance genes. Consequently, *Bacillus licheniformis* DSM 32457 is presumed safe for the target species, consumers of products from animals fed treated silage and the environment.

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 of the additive. Given the proteinaceous nature of the active agent, the additive should be considered a potential respiratory sensitiser.

3.3. Efficacy

The applicant provided data on four laboratory experiments. The applicant claimed that the experiments were performed with easy to ensile forages, as specified by Regulation (EC) No 429/2008.[19] However, this claim was not supported by the analysis of water-soluble carbohydrates in two of the experiments (Guidance on technological additives (EFSA FEEDAP Panel, 2012a)). The FEEDAP Panel noted that endpoints measured at the end of the 90-day experiments were limited to total volatile

[12] Limit of detection (LOD) of the methods for the determination of microbiological contaminants: *Bacillus cereus* 100 CFU/g, Enterobacteriaceae 10 CFU/g, yeast and filamentous fungi 100 CFU/g, *E. coli* not provided.
[13] LOD of the method for the determination of *Salmonella* spp.: 1 CFU/25 g.
[14] Technical dossier/Section II/Annex_II_2.1.4.2.
[redacted]
[17] Technical dossier/Section II/Annex_II_2.1.5.
[redacted]
[19] Technical dossier/Supplementary information March 2019/Section IV.

fatty acids (VFA) and sugars in all four experiments, and dry matter loss in two of them. No measurements of pH, ammonia N, lactic acid, VFA profile were provided for any of the studies. In addition, in one of the studies, the temperature of the silage was measured after exposure to air, but without a comparison with an untreated control. Taking into account the above, the FEEDAP Panel cannot consider any of these studies for the assessment. Therefore, no conclusions can be drawn on the efficacy of *Bacillus licheniformis* DSM 32457.

4. Conclusions

Bacillus licheniformis DSM 32457 is presumed safe for the target species, consumers of products from animals fed treated silage and the environment.

In the absence of data, the FEEDAP Panel cannot conclude on the potential of the additive to be a skin/eye irritant or skin sensitiser. Given the proteinaceous nature of the active agent, the additive should be considered a potential respiratory sensitiser.

In the absence of appropriate data, the FEEDAP Panel cannot conclude on the efficacy of *Bacillus licheniformis* DSM 32457 as a silage additive.

Documentation provided to EFSA/Chronology

Date	Event
23/8/2018	Dossier received by EFSA. Optimize, a preparation of *Bacillus licheniformis* ENV01/DSM 32457 and maltodextrin. Submitted by EnviroSystems (UK) Ltd
7/9/2018	Reception mandate from the European Commission
7/12/2018	Application validated by EFSA – Start of the scientific assessment
6/2/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: Characterisation, Conditions of use and Efficacy*
27/3/2019	Reception of supplementary information from the applicant – Scientific assessment re-started
7/3/2019	Comments received from Member States
25/2/2019	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
3/7/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

CFU	colony-forming unit
DSM	Deutsche Sammlung von Mikroorganismen und Zellkulturen
EURL	European Union Reference Laboratory
FEEDAP	Panel on Additives and Products or Substances used in Animal Feed
LOD	limit of detection
QPS	Qualified Presumption of Safety
VFA	volatile fatty acids

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for *Bacillus licheniformis* DSM 32457

In the current application, authorisation is sought under Article 4(1) for a preparation of *Bacillus licheniformis* ENV01/DSM 32457 (*Optimize*) under the category/functional group 1(k) 'technological additives'/'silage additives', according to Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the *feed additive* in *silage* for all animal species.

According to the Applicant, the *feed additive* contains as active substance viable spores of the non-genetically modified strain *Bacillus licheniformis* ENV01/DSM 32457. The *feed additive* is to be marketed as a powder preparation containing a minimum *Bacillus licheniformis* ENV01/DSM 32457 content of 2.5×10^{10} colony-forming unit (CFU)/g. The *feed additive* is intended to be added, after its reconstitution in water, to silage at a minimum dose of 5×10^{7} CFU/kg of *silage*.

For the identification of *Bacillus licheniformis* ENV01/DSM 32457, the European Union Reference Laboratory (EURL) recommends for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised methodology for genetic identification of bacterial strains.

For the enumeration of *Bacillus licheniformis* ENV01/DSM 32457 in the *feed additive*, 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.

The Applicant did not provide any experimental method or data for the quantification of *Bacillus licheniformis* ENV01/DSM 32457 in silage. Since the unambiguous determination of the content of *Bacillus licheniformis* ENV01/DSM 32457 initially added to *silage* is not achievable by analysis, the EURL cannot evaluate nor recommend any method for official control to quantify the active substance in *silage*.

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.