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SCIENTIFIC OPINION

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Assessment of the feed additive consisting of *Enterococcus lactis* DSM 22502 for all animal species for the renewal of its authorisation (Chr. Hansen A/S)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of *Enterococcus lactis* DSM 22502 as a technological feed additive for all animal species. The applicant provided evidence that the additive currently on the market complies with the existing conditions of authorisation. There is no new evidence that would lead the FEEDAP Panel to reconsider its previous conclusions regarding the safety and efficacy of the additive. The FEEDAP Panel concluded that *E. lactis* remains safe for all animal species, consumers and environment under the authorised conditions of use. Regarding the user safety, the Panel concluded that the additive is not irritating to the skin or eyes. No conclusions can be drawn on the potential of the additive to cause skin sensitisation, but it is considered to be a respiratory sensitiser. There is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

KEYWORDS

E. lactis DSM 22502, renewal, safety, silage additives, technological additives

CONTENTS

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 14(1) of that Regulation lays down that an application for renewal shall be sent to the Commission at the latest one year before the expiry date of the authorisation.

The European Commission received a request from Chr. Hansen A/S[2] for the renewal of the authorisation of the additive consisting of *Enterococcus lactis* DSM 22502,[3] when used as a feed additive for all animal species (category: technological additive; 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 14(1) (renewal of the authorisation). The dossier was received on 12 April 2023 and the general information and supporting documentation are available at https://open.efsa.europa.eu/questions/EFSA-Q-2023-00252. The particulars and documents in support of the application were considered valid by EFSA as of 31 May 2023.

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 feed additive consisting of *Enterococcus lactis* DSM 22502, when used under the proposed conditions of use (see **Section 3.1.4**).

1.2 | Additional information

The additive is a preparation containing *Enterococcus lactis* (formerly identified as *Enterococcus faecium*) DSM 22502 currently authorised as a feed additive for all animal species in the European Union (1k20602). EFSA issued an opinion on the safety and efficacy of this product when used in feed for all animal species (EFSA FEEDAP Panel, 2013).[4]

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[5] in support of the authorisation request for the use of *Enterococcus lactis* DSM 22502 as a feed additive.

In accordance with Article 38 of the Regulation (EC) No 178/2002[6] and taking into account the protection of confidential information and of personal data in accordance with Articles 39 to 39e of the same Regulation, and of the Decision of EFSA's Executive Director laying down practical arrangements concerning transparency and confidentiality,[7] a non-confidential version of the dossier has been published on Open.EFSA.

According to Article 32c(2) of Regulation (EC) No 178/2002 and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations, EFSA carried out a public consultation on the non-confidential version of the technical dossier from 22 December 2023 to 12 January 2024 for which no comments were received.

The confidential version of the technical dossier was subject to a target consultation of the interested Member States from 31 May 2023 to 31 August 2023 for which the received comments were considered for the assessment.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers, other scientific reports and experts' (elicitation) knowledge, to deliver the present output.

[1]Regulation (EC) No 1831/2003 of the European Parliament and of the council of 22 September 2003 on the 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]Originally identified as *Enterococcus faecium*.

[4]Commission Implementing Regulation (EU) No 304/2014 of 25 March 2014 concerning the authorisation of the preparations of *Enterococcus faecium* NCIMB 10415, *Enterococcus faecium* DSM 22502 and *Pediococcus acidilactici* CNCM I-3237 as feed additives for all animal species. OJ L 90, 26.03.2014, p. 8.

[5]Dossier reference: FEED-2022-11015.

[6]Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–48.

[7]Decision available at: https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements

2.2 | Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of *Enterococcus lactis* DSM 22502 is in line with the principles laid down in Regulation (EC) No 429/2008[8] and the relevant guidance documents: Guidance on the renewal of the authorisation of feed additives (EFSA FEEDAP Panel, 2021).

3 | ASSESSMENT

The additive consisting of *Enterococcus lactis* DSM 22502 (formerly identified as *Enterococcus faecium*) is currently authorised as a technological additive (functional group: silage additives) in fresh material for all animal species. The assessment regards the renewal of the authorisation.

3.1 | Characterisation

3.1.1 | Characterisation of the additive

The additive currently authorised is a preparation containing *E. lactis* DSM 22502 at a minimum concentration of 1×10^{11} colony forming units (CFU)/g additive.

The applicant declared that the manufacturing process has not been changed since the previous authorisation. In the current assessment, the applicant provided recent data to characterise the additive as a spray-dried powder consisting of 30% active agent [redacted] maltodextrin, as a carrier, and 8% [redacted], as an anti-caking agent.[9] The qualitative composition of the fermentation media was listed but the quantitative composition was not provided.[10] However, considering the ingredients used, the Panel concluded that none raised safety concerns.

The analysis of five recent and independent batches of the additive showed a mean value of the concentration of *E. lactis* DSM 22502 of 9×10^{10} CFU/g (range: 8.3×10^{10}–1.1×10^{11} CFU/g).[11] The FEEDAP Panel notes that four batches showed values lower than the authorised specifications, but the difference is within 0.5 log, which is considered within the variation of the analytical methods. Therefore, the FEEDAP Panel considers the authorised specifications met.

The applicant also set specifications for *Salmonella* spp. (not detected in 25 g), *Escherichia coli* (< 10 CFU/g), coliforms and total filamentous fungi and yeasts (< 1000 CFU/g) and Enterobacteriaceae (< 10 CFU/g).[11,12] The analysis of five recent and independent batches (three for Enterobacteriaceae) showed compliance with these limits.

Three independent batches of the additive were analysed for the concentration of lead, cadmium, mercury, arsenic and aflatoxin B1. All values were below the limit of quantification (LOQ) of the analytical methods except for mercury (range 0.002–0.005 mg/kg), arsenic (two batches: 0.007 mg/kg) and cadmium (one batch: 0.007 mg/kg).[13]

The FEEDAP Panel considers that the microbial contamination and the amounts of the detected impurities do not raise safety concerns.

3.1.2 | Characterisation of the active agent

The active agent was isolated from faeces of an infant, and it is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DMSZ; Germany) with the accession number DSM 22502.[14] It has not been genetically modified.

The active agent, originally assigned to the *Enterococcus faecium* species (EFSA FEEDAP Panel, 2013), was identified as *Enterococcus lactis* based on a bioinformatic analysis of the whole genome sequence (WGS) data.[15] The taxonomic assignment was based on an average nucleotide identity (ANI) value of 98.69% with the type strain *E. lactis* DSM 23655^T, as compared to an ANI value of 93.77% with the *E. faecium* type strain (DSM 20477^T). [redacted].

The susceptibility of the DSM 22502 strain to antimicrobials was tested using a broth microdilution method and including the set of antibiotics recommended by EFSA (EFSA FEEDAP Panel, 2018).[16] The minimum inhibitory concentration (MIC) values of the strain were compared with the defined EFSA cut-off values for the closest related species *E. faecium*. All the

[8]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.

[9]Sect_II_Identity_E.faecium_DSM22502_ID+Charact_10.2023.

[10]Annex_II_3.1d_Media_ConfMark_v2.

[11]Annex_II_1.3a1_New_CoA_Ef_DSM_22502, Annex_II_1.3a2_New_CoAs_Ef_DSM_22502.

[12]Annex_II_1.3c_Enterobactericeae_Ef_DSM22502.

[13]Annex_II_1.4.2b_Undes.Subs_E.faeciumDSM22502, Annex_II_1.4.2b_Undes.Subs_E.faeciumDSM22502. Limit of quantification (LOQ): < 0.005 mg/kg for cadmium and arsenic, < 0.01 mg/kg for lead, < 0.46 μg/kg for aflatoxin B1.

[14]Annex_II_2.1.2a_Deposit_E.f_DSM22502.

[15]Annex_II_2.1.2b_ID_Ef_DSM 22502, Annex_II_2.2.2a_Gen_Seq_Statement_Ef_DSM22502.

[16]Annex_II_2.2.2c_MIC_Ef_DSM22502.

MIC values were below or equal to the cut-off values, and therefore, the strain is considered to be susceptible to all the relevant antibiotics.

The WGS data of the DSM 22502 strain, [redacted], were interrogated for the presence of antimicrobial resistance (AMR) genes by a search against the NCBI Bacterial Antimicrobial Resistance Reference Gene database [redacted] and ResFinder database [redacted].[17] Three hits were obtained: *eat*(A) (encoding an efflux pump: ABC transporter) [redacted], *msrC* (encoding an efflux pump transporter), and *aac*(6')-Ii (encoding an aminoglycoside 6'-N-acetyltransferase). Genes *mrs(C)* and *aac(6')*-Ii have recently been shown to be intrinsic to *E. lactis* (Lu et al., 2023), and *eat(A)* was already considered intrinsic in *E. faecium* before the splitting of the species in two separate species (Costa et al., 1993; Singh et al., 2001). Therefore, the FEEDAP Panel considers these genes to be of no concern.

The safety of *E. faecium* should be assessed demonstrating the absence of genetic markers typical of the clinical isolates *E. faecium* clade A (IS*16*, *esp*, *hyl*Efm) and the susceptibility to ampicillin. Considering the allocation of clade B strains to *E. lactis* species, the FEEDAP Panel considers these criteria are also applicable to *E. lactis* strains.[18] *E. lactis* DSM 22502 was susceptible to ampicillin (MIC 1–2 mg/L) and none of the three genetic determinants were detected by BLASTn analysis of the WGS data.

3.1.3 | Physical properties of the additive

Considering that no changes have been introduced in the manufacturing process and composition, the data on physicochemical properties described in the previous opinion (EFSA FEEDAP Panel, 2013) are still valid.

However, in the current assessment, the applicant provided new data on particle size distribution. The particle size of the additive was analysed by laser diffraction method in three batches; the results showed an average 8.62% (range 8.37%–9.07%) for the fraction (v/v) < 10 μm, 52.05% (range 51.56%–52.96%) for the fraction < 50 μm and 80.73% (range 80.41%–81.22%) for the fraction < 100 μm.[19]

3.1.4 | Conditions of use

The additive is currently authorised for use as a silage additive in fresh material for all animal species.[20] Under those provisions of the authorisation,[21] it is specified that:

- In the directions for use of the additive and premixture, indicate the storage temperature and storage life.
- Minimum dose of the additive when it is not used in combination with other microorganisms as silage additive: 1×10^8 CFU/kg of fresh material.
- For safety: It is recommended to use breathing protection and gloves during handling.

The applicant intends to maintain the same conditions of use as in the authorisation.

3.2 | Safety

In the previous opinion (EFSA FEEDAP Panel, 2013), it was concluded: 'None of the four *Enterococcus faecium* strains (NCIMB 14015, DSM 22502, ATTC 53510 and ATTC 55593) was shown to contain marker genes typical of hospital-associated isolates responsible for clinical infections and all were susceptible to clinically relevant antibiotics. In addition, no other sources of concern have been identified in the additives. Consequently, the FEEDAP Panel considers the use of these *E. faecium* strains as silage additives safe for consumers of animal products. It is not expected that the use of *E. faecium* at the doses proposed would substantially increase the exposure of animals given silage as part of their rations. Therefore, the FEEDAP Panel considers that the use of these strains in the preparation of silage is safe for the target animals. […] The use of these strains as silage additives is considered safe for the environment.'

The applicant declared that no incidents or safety issues for target animals, consumers, users and/or the environment have been documented or reported regarding the additive since its first authorisation.[22]

In the context of the current application, the identity of DSM 22502 strain was reassigned to *E. lactis*, and evidence was provided that the strain does not harbour acquired AMR genes or it is virulent. The FEEDAP Panel considers the criteria to assess

[17]Annex_II_2.2.2b_Gen_AMR_Ef_DSM22502.

[18]Annex_II_2.2.2e_Pathogenicity_Statement_DSM22502.

[19]Annex_II_1.5_Particle_size_distr_Ef_DSM22502.

[20]Sect_II_Identity_E.faecium_DSM22502_Cond_of_use.

[21]Commission Implementing Regulation (EU) No 304/2014 of 25 March 2014 concerning the authorisation of the preparations of *Enterococcus faecium* NCIMB 10415, *Enterococcus faecium* DSM 22502 and *Pediococcus acidilactici* CNCM I-3237 as feed additives for all animal species. OJ L 90, 26.03.2014, p. 8.

[22]Sect_III_Safety_E.faecium_DSM22502_User, Statement_DSM22502_2023.

the safety of *E. faecium* applicable also to *E. lactis* strains. In addition, the manufacturing process of the additive, its composition and the conditions of use have not been modified. Consequently, the conclusions previously reached are still deemed valid, and the Panel considers that *E. lactis* DSM 22502 remains safe for the target species, consumers and the environment.

In support of the safety of the additive, the applicant submitted the results of a literature search performed in accordance with the requirements of the Guidance on the renewal of the authorisation of feed additives (EFSA FEEDAP Panel, 2021).[23] The search period covered the full period since the authorisation. Four databases were searched (Academic Onefile, food Science Source, AGRIS and PubMed). A total of 203 references were retrieved after excluding the duplicates. After a first screening, six references were selected for full text review. This resulted in a final selection of three scientific papers. None of them provided information relevant to the safety assessment of the additive under assessment.

In the previous opinion (EFSA FEEDAP Panel, 2013), the Panel also evaluated the safety for the users and concluded: 'In the absence of evidence, these additives[24] should be regarded as skin and eye irritants and potential skin sensitisers. Given the proteinaceous nature of the active agents, the FEEDAP Panel considers it prudent to treat these additives as respiratory sensitisers. Given the high dusting potential of most of the preparations tested, there is a need to take measures to minimise inhalation exposure of workers.'

For the current evaluation, no specific studies were submitted on the additive under assessment with regard to the safety for the user. However, the applicant referred to an in vivo skin irritation study (according to OECD Guideline 404), two in vitro eye irritation studies (according to OECD Guideline 492 and OECD Guideline 438, respectively) and an in vivo skin sensitisation study (according to OECD Guideline 429) performed with a test item containing the active agent under assessment and sorbitol as a carrier.[25] The studies performed with this test item did not indicate a potential for irritation of the skin and eyes; the FEEDAP Panel considers that the tests can be used to conclude that the additive under assessment is not irritant to skin and eyes.

The same test item was indicated as a skin sensitiser, based on the studies submitted. However, the FEEDAP Panel notes that the OECD test guidelines available at present are designed to assess the skin sensitisation potential of chemical substances only and that currently no validated assays for assessing the sensitisation potential of microorganisms are available. Therefore, no conclusions can be drawn on the potential of the additive to cause skin sensitisation.

Moreover, owing to the proteinaceous nature of the active agent, the additive should be considered a respiratory sensitiser.

3.2.1 | Conclusions on safety

The FEEDAP Panel concludes that the additive is safe for the target animal species, consumers and the environment under the authorised conditions of use. The additive under assessment is considered not irritant to skin or eyes but is a respiratory sensitiser. No conclusions can be drawn on the potential of the additive to cause skin sensitisation.

3.3 | Efficacy

The present application for renewal of the authorisation does not include a proposal for amending or supplementing the conditions of the original authorisation that would have an impact on the efficacy of the additive. Therefore, there is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

4 | CONCLUSIONS

The applicant has provided evidence that the additive currently on the market complies with the existing conditions of authorisation.

The Panel concludes that *Enterococcus lactis* DSM 22502 remains safe for all target animal species, consumers and the environment under the authorised conditions of use.

The additive is not irritating to the skin or eyes but is considered a respiratory sensitiser. No conclusion could be drawn on the skin sensitisation potential of the additive.

There is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

ABBREVIATIONS

AMR antimicrobial resistance
CFU colony forming unit
FEEDAP EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
LOQ limit of quantification
MIC minimum inhibitory concentration

[23] Annex_III_1a_Flow_diagram_Lit.search_E.faecium.silage_2022–23, Annex_III_1c_Search_Method_Description_E.faecium.silage.

[24] Silage additives assessed in EFSA FEEDAP Panel, 2013: *Enterococcus faecium* NCIMB 14015, DSM 22502, ATTC 53510 and ATTC 55593.

[25] Sect_III_Safety_E.faecium_DSM22502_User, Certificate_of_Composition_LWS_Updated, Annex_III_3_1a_115–404-7124_SkinIrr, Annex_III_3_1b_115–492-6704_EyeIrr_InVitro1, Annex_III_3_1c_115–438-7162_EyeIrr_InVitro2, Annex_III_3_1d_115–429-6705_SkinSens.

OECD Organisation for Economic Co-operation and Development
WGS whole genome sequence

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CONFLICT OF INTEREST
If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

REQUESTOR
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