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SCIENTIFIC OPINION

Assessment of the feed additive consisting of *Pediococcus pentosaceus* DSM 23688 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 assessment of the application for renewal of *Pediococcus pentosaceus* DSM 23688, a technological additive for all animal species. The applicant has provided evidence that the additive currently on the market complies with the existing conditions of authorisation. The Panel concluded that the additive remains safe for all animal species, consumers, and the environment under the authorised conditions of use. Regarding user safety, the additive was shown not to be irritant to skin or eyes. The Panel was not in the position to conclude on skin sensitisation potential of the additive, 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

Pediococcus pentosaceus DSM 23688, QPS, renewal, safety, silage, technological additive

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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 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 *Pediococcus pentosaceus* DSM 23688, 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 20 February 2023 and the general information and supporting documentation are available at https://open.efsa.europa.eu/questions/EFSA-Q-2023-00163. The particulars and documents in support of the application were considered valid by EFSA as of 31 August 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 *Pediococcus pentosaceus* DSM 23688, when used under the proposed conditions of use (see **Section 3.1.3**).

1.2 | Additional information

The additive *P. pentosaceus* DSM 23688 is currently authorised for use in feed for all animal species (1k1010).[3]

EFSA issued an opinion on the safety and efficacy of this product when used in feed for all animal species (EFSA FEEDAP Panel, 2013).

2 | DATA AND METHODOLOGIES

2.1 | Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[4] in support of the authorisation request for the use of *P. pentosaceus* DSM 23688 as a feed additive.

The confidential version of the technical dossier was subject to a target consultation of the interested Member States from 31 August 2023 to 1 December 2023; the comments received were considered for the assessment.

In accordance with Article 38 of the Regulation (EC) No 178/2002[5] 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,[6] 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 06 December 2023 to 27 December 2023 for which no comments were received.

The European Union Reference Laboratory (EURL) considered that the conclusions and recommendations reached in the previous assessment regarding the methods used for the control of *P. pentosaceus* DSM 23688 in animal feed are valid and applicable for the current application.[7]

[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]Commission Implementing Regulation (EU) No 84/2014 of 30 January 2014 concerning the authorisation of preparations of *Pediococcus pentosaceus* DSM 14021, *Pediococcus pentosaceus* DSM 23688 or *Pediococcus pentosaceus* DSM 23689 as feed additives for all animal species. OJ L 28, 31.1.2014, p. 30.

[4]Dossier reference: FEED-2022-11013.

[5]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.

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

[7]Evaluation report available on the EU Science Hub https://joint-research-centre.ec.europa.eu/system/files/2013-02/FinRep-FAD-uorg3.pdf

2.2 | Methodologies

The approach followed by the FEEDAP Panel to assess the efficacy of *Pediococcus pentosaceus* DSM 23688 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 *P. pentosaceus* DSM 23688 is currently authorised for all animal species as a technological additive (functional group: silage additives). The assessment regards the renewal of the authorisation.

3.1 | Characterisation

3.1.1 | Characterisation of the additive

The additive is currently authorised with a minimum content of the active agent (*P. pentosaceus* DSM 23688) of 1×10^{11} colony forming units (CFU)/g additive. The applicant declared that the manufacturing process has not been modified since the previous authorisation and that no antimicrobials are used during the manufacturing process.[9] The qualitative composition of the fermentation medium was listed but the quantitative composition was not provided.[10] However, considering the ingredients used, the Panel concluded that none raised safety concerns.

The final product is a powder containing the freeze-dried cell concentrate (17%–42%; including a maximum of [redacted], 3% water and [redacted]), [redacted][11] as an anti-caking agent (8%) and [redacted] as a carrier (50%–75%), to reach the minimum concentration specified for *P. pentosaceus* DSM 23688.[12]

Analytical data to confirm the specification set as per authorisation was provided for five independent batches of the additive, showing an average value of 4.6×10^{11} CFU/g additive (range $2.9–8.1 \times 10^{11}$ CFU/g).[13]

Three batches of the additive were analysed for cadmium, lead, mercury, arsenic and aflatoxin B1 concentrations. The following range of values were obtained: cadmium 0.020–0.039 mg/kg, lead 0.023–0.033 mg/kg, mercury 0.0047–0.0221 mg/kg, arsenic 0.028–0.037 mg/kg. The results for aflatoxin B1 were below the limit of quantification (LOQ) of the method.[14,15]

Five batches showed compliance with the specifications set for coliforms (< 1000 CFU/g), *Escherichia coli* (< 10 CFU/g), yeasts and filamentous fungi (< 1000 CFU/g) and *Salmonella* spp. (not detected in 25 g frozen product (one batch) or 5 g freeze dried bulk (four batches)).[16] Additionally, three batches showed compliance with the specifications set for Enterobacteriaceae (< 10 CFU/g).[17]

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

No new data were provided regarding the physico-chemical properties or stability of the additive. Since no changes were introduced in the manufacturing process and composition, the data described in the previous opinion (EFSA FEEDAP Panel, 2013) still apply.

3.1.2 | Characterisation of the active agent

The active agent was originally isolated from silage, and it is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM) with the accession number DSM 23688.[18] It has not been genetically modified.

The taxonomical identification of the active agent as *P. pentosaceus* was confirmed by Average nucleotide identity (ANI) determination using the whole genome sequence (WGS).[19] The ANI value obtained was 98.1% with the type strain *P. pentosaceus* ATCC 33316^T.[20]

[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]Statement_DSM23688_2023_ConfMark.
[10]Annex_II_3.1d_GrossIngrList_Updated_Nov2023.
[11]Currently under re-evaluation by the FEEDAP Panel.
[12]Sect_II_Identity_P.pen_DSM23688_ID+Charact_v3, Supplementary_confidential_Info_DSM23688_12.2023 and Annex_II_3.1d_GrossIngrList_Updated_Nov2023.
[13]Annex_II_1.3a_CoAs_new_DSM23688_v1 and Annex_II_1.3a_CoAs_new_DSM23688_v2.
[14]Annex_II_1.4.2_undes.subst_DSM23688_v1 and Annex_II_1.4.2_undes.subst_DSM23688_v2.
[15]LOQ for aflatoxin B1: 46 µg/kg.
[16]Annex_II_1.3a_CoAs_new_DSM23688_v1 and Annex_II_1.3a_CoAs_new_DSM23688_v2.
[17]Annex_II_1.4.2a_Enterobacteriaceae_DSM23688.
[18]Annex_II_2.1.2a_Deposit_proof_DSM23688 + DSM23689.
[19]Annex_II_2.2.2a_DSM23688_GenSeqState.
[20]Annex_II_2.1.2b_DSM_23688_ID.

The susceptibility of *P. pentosaceus* DSM 23688 to antimicrobials was tested using a broth microdilution method and including the antimicrobials recommended by EFSA (EFSA FEEDAP Panel, 2018).[21] All the minimum inhibitory concentration values were equal to or below the corresponding cut-off values, except for tetracycline, which was one dilution step above the cut-off value (16 vs. 8 mg/L). 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. Therefore, the strain is considered to be susceptible to all the relevant antibiotics.

The WGS data were interrogated for the presence of antimicrobial resistance (AMR) genes against the ResFinder and NCBI Bacterial Antimicrobial Resistance Reference Gene databases, at nucleotide and protein level respectively, [redacted]. Thresholds were set at [redacted] identity and [redacted] coverage, and no hits of concern were identified.[22]

3.1.3 | Conditions of use

The additive is currently authorised as a silage additive for all animal species. Under other provisions of the authorisation, it is specified that:

- In the directions for use of the additive and premixture, indicate the storage conditions.
- Minimum content of the additive when used without combination with other microorganisms as silage additives: 1×10^8 CFU/kg fresh material in easy and moderately difficult to ensile material.
- For safety: it is recommended to use breathing protection, eye protection and gloves during handling.

The applicant intends to maintain the same conditions of use since the authorization.[23]

3.2 | Safety

In its previous opinion the FEEDAP Panel concluded that, following the qualified presumption of safety (QPS) approach to the safety assessment, *P. pentosaceus* DSM 23688 is safe for the target species, consumers and the environment (EFSA FEEDAP Panel, 2013). Regarding user safety, the Panel concluded that '*No data were provided on skin/eye irritancy or skin sensitisation for any of the additives.*[24] *However, the generic material safety data sheet proposed indicates that preparations containing the strains may cause irritation on prolonged contact with skin and eyes. The dusting potential of commercial formulations tested was high. This, coupled with the significant fraction of these products that is potentially inhalable, means that exposure via a respiratory route is a significant possibility and hazard… given the proteinaceous nature of the active agents, the additives should be considered to have the potential to be respiratory sensitisers and treated accordingly*'.

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 approval.[25]

In the context of the current application, the identity of the strain as *P. pentosaceus* was confirmed, and evidence that the strain does not show antimicrobial resistance for antibiotics of human and veterinary importance was provided (EFSA BIOHAZ Panel, 2023). Consequently, the conclusions previously reached are still valid, and the Panel considers that *P. pentosaceus* DSM 23688 remains safe for the target species, consumers and the environment.

An extensive literature search was performed to support the safety of the *P. pentosaceus* species and the *P. pentosaceus* DSM 23688 strain. The search was performed in December 2022 and the terms used included the active agent at strain level and the commercial name of the product. The applicant searched a total of four databases: Academic OneFile, Food Science Source, AGRIS and PubMed. The literature search retrieved 95 hits. None of the articles described safety concerns related to the use of *P. pentosaceus* strains.[26]

Regarding the safety for the user, the applicant provided for the current evaluation the following three new studies using the additive as the test item.

The skin irritation potential of the additive was investigated in vivo using New Zealand White rabbits according to OECD TG 404.[27] The results of the study showed that the additive is classified as non-irritant to the skin (UN GHS 'No Category').

The eye irritation potential of the additive was investigated in an in vitro eye irritation study according to OECD TG 492.[28] Based on the results of the study the additive is considered non-irritant to eyes (UN-GHS 'No Category').

[21] Annex_II_2.2.2c_MIC_DSM23688.
[22] Annex_II_2.2.2b_DSM23688_Genome AMR report.
[23] Sect_II_Identity_P.pen_DSM23688_Cond_of_use.
[24] Silage additives assessed in EFSA FEEDAP Panel, 2013: *Pediococcus pentosaceus* DSM 14021, DSM 23688 and DSM 23689.
[25] Sect_III_Safety_P.pen_DSM23688_User and Statement_DSM23688_2023.
[26] Annex_III.1a_flow_diagram_Lit.search_P.pen_2022 and Annex_III.1c_Search_Method_Description_P.pen_2022.
[27] Annex_III_3.1b_404_SkinIrr_InVivo.
[28] Annex_III_3.1a_492_Eye_irr_InVitro.

The local lymph node assay was performed to assess the skin sensitisation potential of the additive following the OECD TG 429.[29] The additive was shown to have no skin sensitisation potential in this assay. 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.

3.2.1 | Conclusions on safety

The FEEDAP Panel concludes that *P. pentosaceus* DSM 23688 remains safe for the target animal species, consumers and the environment under the authorised conditions of use. The additive is not irritant to skin or eyes but is considered to be 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 terms of authorisation.

The Panel concludes that *P. pentosaceus* DSM 23688 remains safe for all target animal species, consumers and the environment under the authorised conditions of use. Regarding the safety for the user, the additive is not irritant to skin or eyes, but is considered to be a respiratory sensitiser. The Panel is not in the position to conclude 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
DSM	Deutsche Sammlung von Mikroorganismen und Zellkulturen
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
LOQ	limit of quantification
MIC	minimum inhibitory concentration
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

European Commission

QUESTION NUMBER

EFSA-Q-2023-00163

[29] Annex_III_3.1c_429_SkinSens_InVivo.

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