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# Safety and efficacy of *Pediococcus pentosaceus* DSM 32291 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 was asked to deliver a scientific opinion on the safety and efficacy of a strain of *Pediococcus pentosaceus* when used as a technological additive intended to improve ensiling at a proposed application rate of $5 \times 10^7$ CFU kg/fresh matter. The species *P. pentosaceus* is considered by EFSA to be suitable for the qualified presumption of safety approach to safety assessment and not to require specific demonstration of safety other than the absence of resistance to antibiotics of human and veterinary significance. As the identity of the strain was clearly established and as no antibiotic resistance was detected, the use of the strain in the production of silage is presumed safe for livestock species, consumers of products from animals fed treated silage and the environment. In the absence of data, no conclusion can be drawn on the skin and eye irritancy of the additive. The additive should be considered a potential respiratory sensitiser. *Pediococcus pentosaceus* DSM 32291 at a minimum dose of $5 \times 10^7$ CFU/kg has the potential to improve the production of silage from easy and moderately difficult to ensile materials by decreasing dry matter loss and protein degradation during ensiling.



**Keywords:** technological additive, silage additive, *Pediococcus pentosaceus*, safety, QPS, 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 Microferm Limited[2] for authorisation of the product *Pediococcus pentosaceus* DSM 32291, 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). EFSA received directly from the applicant the technical dossiers in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 3 May 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 *Pediococcus pentosaceus* DSM 32291, 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 *P. pentosaceus* DSM 32291. It has not been previously authorised as a feed additive in the European Union.

The species *P. pentosaceus* 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.

# 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 *Pediococcus pentosaceus* DSM 32291 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 substance 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 *Pediococcus pentosaceus* DSM 32291 is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on technological additives (EFSA FEEDAP Panel, 2012a), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b) and Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA FEEDAP Panel, 2012c).

[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] Microferm Limited, Spring Lane North, Malvern Link WR141BU Worcestershire United Kingdom.
[3] FEED dossier reference: FAD-2017-0025.
[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: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2017-0025-pediococcus-pent.pdf

# 3. Assessment

The present additive is based on a preparation of a single strain of *P. pentosaceus* and is intended to be added to forages to promote ensiling (technological additive, functional group: silage additive) with the eventual use of the silage in all animal species.

## 3.1. Characterisation

### 3.1.1. Characterisation of the active agent

The strain of *P. pentosaceus* was originally isolated from cut grass and is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) with the accession number DSM 32291.[6] It has not been genetically modified. Identity was established by its phenotypic properties and by the full 16S rRNA gene sequence (1477 bp) and by multilocus sequence typing based on four housekeeping genes (*rpoA, pheS, atpA and dnaK*) which by comparison with sequences recorded in databases gave an unambiguous identification.[7]

Genetic stability was examined by comparison of the master culture with three consecutive working cultures using random amplification of polymorphic DNA polymerase chain reaction (RAPD-PCR).[8] No significant differences in the resultant RAPD patterns were observed.

The strain was tested for antibiotic susceptibility using a serial dilution method.[9] The battery of antibiotics tested included those recommended by EFSA (EFSA FEEDAP Panel, 2012c). All of the minimum inhibitory concentration values for the *P. pentosaceus* strain were equal to or fell below the cut-off values defined by the FEEDAP Panel. Consequently, the strain is considered to be susceptible to all relevant antibiotics.

### 3.1.2. Characterisation of the product[10]

The manufacturing process is detailed in the dossier. The additive is produced with a minimum declared content of $8 \times 10^{10}$ CFU/g. Analysis of five cell batches gave a mean viable count of $6.7 \times 10^{11}$ CFU/g (range 5.6–7.6 $\times$ $10^{11}$ CFU/g).[11]

Each batch of *P. pentosaceus* DSM 32291 received from the producer is routinely examined for total coliforms, *Escherichia coli*, *Salmonella* spp., total yeasts and filamentous fungi. No microbial contaminants were detected in five batches (< 10 CFU/g or absence of *Salmonella* in 1 g).[12]

Five batches of the additive[13] and three batches of corn steep liquor[14] used in the growth medium were examined for the presence of heavy metals (Cd, Pb and Hg), arsenic and aflatoxins B1, B2, G1 and G2. In all cases, heavy metals and arsenic were found only in trace amounts (< 0.1 mg/kg), except for lead and arsenic where values of corn steep liquor samples were $\leq$ 0.4 mg/kg and $\leq$ 0.17 mg/kg, respectively. Aflatoxins were not detected (< 0.1 μg/kg) in the corn steep liquor, but were found at levels $\leq$ 0.16 μg/kg in the batches of *P. pentosaceus* DSM 32291. Based on these results, routine analyses to detect the presence of heavy metals, arsenic and aflatoxins are not applied to the batches provided by the producer.

No specific data were provided on the particle size distribution or dusting potential of the additive under assessment.

### 3.1.3. Stability

Three batches of the additive were standardised to a count of $1 \times 10^{11}$ CFU/g using maltodextrin as a carrier and a further three batches were standardised to a count of $2.5 \times 10^{10}$ CFU/g using glucose.[15] The samples were stored in aluminium foil bags at room temperature. Losses were < 0.5 log after 18 months storage for both formulations.

[6] Technical dossier/Section II/Annex II.8.
[7] Technical dossier/Section II/Annexes II.7.
[8] Technical dossier/Section II/Annex II.9.
[9] Technical dossier/Section II/Annex II.10.
[10] This section has been edited following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.
[11] Technical dossier/Supplementary information October 2017.
[12] Technical dossier/Section II/Annex II.3.
[13] Technical dossier/Section II/Annex II.1.
[14] Technical dossier/Section II/Annex II.2.
[15] Technical dossier/Section II.

*Pediococcus pentosaceus* DSM 32291 was standardised to a count of $1 \times 10^{11}$ CFU/g using glucose as a carrier and including diammonium phosphate (5%) and dipotassium phosphate (2.5%) as buffers. Three samples (each of 5 g) were suspended in 1 L water giving a count of $5 \times 10^{8}$ CFU/mL and stored for 7 days at room temperature. No loss of viability was detected after 3 days and even after 7 days losses were $\leq$ 0.2 log of the initial value.

#### 3.1.4. Conditions of use

The additive is intended for use with easy and moderately difficult to ensile forages for all animal species at a proposed minimum dose of $5 \times 10^{7}$ CFU/kg forage.[11] It can be applied dry or dispersed in water.

### 3.2. Safety

#### 3.2.1. Safety for the target species, consumers and environment

In the view of the FEEDAP Panel, the identity of the strain has been established and the antibiotic resistance qualification met. Consequently, *Pediococcus pentosaceus* DSM 32291 is considered to be suitable for the QPS approach to safety assessment and 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 data were submitted on skin/eye irritation or skin sensitisation. Therefore, no conclusions can be drawn on the skin and 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.

Once an active agent has been authorised as a silage additive, different formulations can be placed on the market with reference to that authorisation. The applicant indicated that the additive could be incorporated into a number of different formulations and, consequently, not all forms can be directly tested for user safety. However, for assessing the safety for the user of the additive, the active agent is the principal concern provided that other components do not introduce safety issues. For this specific product, the excipients described do not introduce additional risks.

### 3.3. Efficacy

Three laboratory experiments were made with different forage samples representing materials easy to ensile (study 1) and moderately difficult to ensile (studies 2 and 3), as specified by Regulation (EC) No 429/2008 (Table 1).[16]

In all the studies, forage was ensiled in mini-silos with a capacity of 4.5 L. All of the silos were fitted with air-locks to vent gas. The additive was dissolved in water and sprayed on the forage at an intended concentration of $5 \times 10^{7}$ CFU/kg fresh matter (not confirmed by analysis).

Forage for the control silos were sprayed with an equal volume of water, but without the additive. Four replicate silos were prepared for each experimental treatment (with or without the additive). The ambient temperature during ensiling was controlled at 20 $\pm$ 2°C and the duration of the experiments was 90 days.

**Table 1:** Characteristics of the forage samples used in the ensiling experiments

| Study | Test material | Dry matter content (%) | Water-soluble carbohydrate content (% fresh matter) |
|---|---|---|---|
| 1 | Grass/herb mixture (95:5)[(a)] | 38.1 | 4.7 |
| 2 | Grass/red clover/herb mixture (75:20:5)[(b)] | 19.8 | 2.5 |
| 3 | Grass/red clover/herb mixture (70:25:5)[(c)] | 24.9 | 2.6 |

(a): Grasses were predominately timothy, perennial ryegrass and a *Lolium multiflorum* x *Festuca arundinacea* hybrid.
(b): Grasses were mainly timothy, perennial ryegrass and orchard grass.
(c): Grasses were mainly timothy, meadow fescue and perennial ryegrass.

[16] Technical dossier/Section IV/Annexes IV.1 and IV.2.

Silos were opened at the end of the experiment and the contents were analysed to determine dry matter (DM) content, pH, lactic and volatile fatty acids concentrations, ammonia, ethanol, 1,2-propanediol and 2,3 butanediol. DM loss during ensiling was calculated. Statistical evaluation of data was by a non-parametric test (Wilcoxon Kruskal–Wallis test), comparing treated versus control silos.[17] Significance was declared at $p < 0.05$.

**Table 2:** Summary of the analysis of ensiled material recovered at the end of the ensiling period with *Pediococcus pentosaceus* DSM 32291

| Study | Application rate (CFU/kg forage) | Dry matter loss (%) | pH | Lactic acid (% fresh matter) | Acetic acid (% fresh matter) | Ammonia-N (% total N) |
|---|---|---|---|---|---|---|
| 1 | 0 | 3.2 | 5.0 | 1.8 | 0.3 | 6.4 |
| | $5 \times 10^7$ | 2.5* | 4.2* | 2.9* | 0.1* | 5.2* |
| 2 | 0 | 10.4 | 5.2 | 0.7 | 0.3 | 21.5 |
| | $5 \times 10^7$ | 2.6* | 4.0* | 1.9* | 0.1* | 5.6* |
| 3 | 0 | 8.0 | 5.4 | 0.5 | 0.2 | 16.0 |
| | $5 \times 10^7$ | 1.8* | 4.0* | 2.1* | 0.1* | 5.6* |

CFU: colony forming unit.
*: Significantly different from the control value at $p < 0.05$.

In the three studies, dry matter loss and ammonia-N as percentage of total N were reduced when forage was treated with the additive, suggesting an improved production of silage (Table 2). The significant decrease in silage pH and increase in lactic acid concentration in treated silage would indicate an improved fermentation in silage treated with the additive. Therefore, the additive has the potential to improve the production of silage from easy and moderately difficult to ensile materials.

## 4. Conclusions

As the identity of the strain *Pediococcus pentosaceus* DSM 32291 has been established and no antibiotic resistance of concern was detected, following the QPS approach to safety assessment, the use of this strain in the production of silage is presumed safe for target species, consumers of products from animals fed treated silage and for the environment.

In the absence of data, no conclusion can be drawn on the skin and eye irritancy of the additive. The additive should be considered a potential respiratory sensitiser.

*Pediococcus pentosaceus* DSM 32291 at a minimum dose of $5 \times 10^7$ CFU/kg has the potential to improve the production of silage from easy and moderately difficult to ensile forage materials by decreasing DM loss and protein degradation during ensiling.

## Documentation provided to EFSA

1) *Pediococcus pentosaceus* DSM 32291. April 2017. Submitted by Microferm Limited.
2) *Pediococcus pentosaceus* DSM 32291. Supplementary information October 2017. Submitted by Microferm Ltd.
3) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for *Pediococcus pentosaceus* DSM 32291.

## References

EFSA (European Food Safety Authority), 2007. Opinion of the Scientific Committee on a request from EFSA on the introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. EFSA Journal 2007;5(12):587, 16 pp. Available online: https://www.efsa.europa.eu/en/efsajournal/pub/587

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2017. Ricci A, Allende A, Bolton D, Chemaly M, Davies R, Girones R, Herman L, Koutsoumanis K, Lindqvist R, Nørrung B, Robertson L, Ru G, Sanaa M, Simmons M, Skandamis P, Snary E, Speybroeck N, Ter Kuile B, Threlfall J, Wahlström H, Cocconcelli PS, Klein G (deceased), Prieto Maradona M, Querol A, Peixe L, Suarez JE, Sundh I, Vlak JM, Aguilera-Gomez M, Barizzone F, Brozzi R, Correia S, Heng L, Istace F, Lythgo C and Fernández Escámez PS, 2017. Scientific Opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA. EFSA Journal 2017;15(3):4664, 177 pp. https://doi.org/10.2903/j.efsa.2017.4664

[17] Technical dossier/Section IV/Annex IV.3.

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012a. Guidance for the preparation of dossiers for technological additives. EFSA Journal 2012;10(1):2528, 23 pp. https://doi.org/10.2903/j.efsa.2012.2528

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012b. Guidance on studies concerning the safety of use of the additive for users/workers. EFSA Journal 2012;10(1):2539. 5 pp https://doi.org/10.2903/j.efsa.2012.2539

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012c. Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. EFSA Journal 2012;10(6):2740, 10 pp. https://doi.org/10.2903/j.efsa.2012.2740

## Abbreviations

| | |
|---|---|
| CFU | colony forming unit |
| DM | dry matter |
| DSMZ | Deutsche Sammlung von Mikroorganismen und Zellkulturen |
| EURL | European Union Reference Laboratory |
| FEEDAP | EFSA Panel on Additives and Products or Substances used in Animal Feed |
| PFGE | pulsed field gel electrophoresis |
| RAPD-PCR | random amplification of polymorphic DNA polymerase chain reaction |
| QPS | qualified presumption of safety |

# Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for *Pediococcus pentosaceus* DSM 32291

In the current application authorisation is sought under Article 4(1) for *Pediococcus pentosaceus* DSM 32291 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 cells of the strain *Pediococcus pentosaceus* DSM 32291. The *feed additive* is to be marketed as a preparation containing a minimum *Pediococcus pentosaceus* DSM 32291 content of $8 \times 10^{10}$ Colony Forming Units (CFU)/g. The *feed additive* is intended to be added dry or wet via a water suspension to silage at a minimum dose of $5 \times 10^{7}$ CFU/kg fresh *silage.*

For the identification of *Pediococcus pentosaceus* DSM 32291, the EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised methodology for microbial identification.

For the enumeration of *Pediococcus pentosaceus* DSM 32291 in *feed additive,* the Applicant submitted the ring-trial validated spread plate method EN 15786:2009. 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 *Pediococcus pentosaceus* DSM 32291 in *silage.* Since the unambiguous determination of the content of *Pediococcus pentosaceus* DSM 32291 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.