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Safety and efficacy of *Pediococcus parvulus* DSM 28875 as a silage additive for all animal species

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Abstract

The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of a specific strain of *Pediococcus parvulus* when used as a technological additive intended to improve ensiling at a proposed application rate of 1×10^8 CFU/kg fresh matter. The species *P. parvulus* 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 or skin sensitisation of the additive. The additive should be considered as a potential respiratory sensitiser. Five studies with laboratory-scale silos were made using forage of differing water-soluble carbohydrate content. Replicate silos containing forages treated at the proposed application rate were compared to identical silos containing the same but untreated forage. The minisilos were then stored for 90–103 days at 20–24°C. After opening, the contents of the silos were analysed. Results showed that *Pediococcus parvulus* DSM 28875 at 5×10^7 CFU/kg forage has the potential to improve the production of silage from easy and moderately difficult to ensile forage species by reducing dry matter loss and enhancing protein preservation.

Keywords: technological additive, silage additive, *Pediococcus parvulus*, 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 Ltd[2] for authorisation of the product *Pediococcus parvulus* DSM 28875, when used as a feed additive for all animals species (category: technological additives; functional group: silage additives).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded to the European Food Safety Authority (EFSA) 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 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 parvulus* DSM 28875, 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 *Pediococcus parvulus* DSM 28875. It has not been previously authorised as a feed additive in the European Union (EU).

The species *P. parvulus* is considered by EFSA to be suitable for the Qualified Presumption of Safety (QPS) approach to safety assessment (EFSA, 2007, EFSA BIOHAZ Panel, 2013). 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 parvulus* DSM 28875 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 parvulus* DSM 28875 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), Technical guidance on the tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011), Guidance on studies concerning the safety of use of the additive for

[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 Ltd, Spring Lane North, Malvern Link WR141BU Worcestershire, United Kingdom.
[3] FEED dossier reference: FAD-2016-0015.
[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_fad20160015_pediococcus_p.pdf

users/workers (EFSA FEEDAP Panel, 2012b) and Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA, 2012c).

3. Assessment

Six genera of lactic acid-producing bacteria are commonly associated with forage species and collectively contribute to the natural ensiling process. The present additive is based on a preparation of a single strain of one of those six genera, *P. parvulus*, 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 any animal species.

3.1. Characterisation

3.1.1. Characterisation of the active agent

The strain of *P. parvulus* was isolated from grass and is deposited with the German Collection of Microorganisms and Cell Cultures (DSMZ) with the accession number DSM 28875.[6] It has not been genetically modified. Species identity was established by analysis of the full 16S rRNA gene sequence[7] and multilocus sequence (MLS) typing targeted to *rpoA*, *pheS, dnaK* and *atpA* genes.

Genetic stability was examined by comparison of the random amplification of polymorphic DNA (RAPD) profile of the master culture with three consecutive working cultures and the culture collection stock. No differences in the resultant patterns were observed.[8]

The strain was tested for antibiotic susceptibility using a serial twofold dilution procedure in liquid medium. The battery of antibiotics tested was that recommended by EFSA (EFSA, 2012c).[9] The minimum inhibitory concentration (MIC) values of kanamycin, gentamycin, streptomycin, clindamycin and erythromycin did not exceed the corresponding cut-off values defined by the FEEDAP Panel. The MIC values of ampicillin, tetracycline and chloramphenicol were higher than the defined cut-off values. As the MICs for these antibiotics were within one dilution step of the cut-off value and within the normal variation, no further investigation is considered necessary and 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.

Analysis of five production batches showed that counts exceeded the specification in all cases (mean value of 5.5×10^{11} CFU/g additive, range 4.5–6.6 $\times$ 10^{11} CFU/g additive).

The additive is routinely monitored for microbial contamination at every batch. Limits are set for yeasts and filamentous fungi (< 10 CFU/g), presumptive coliforms and *Escherichia coli* (< 10 CFU/g) and *Salmonella* spp. (absent in 25 g). Compliance with specifications was proved in five batches.[11]

Heavy metals (Pb, Cd and Hg) and arsenic were also analysed in five batches of the product. Arsenic and lead were < 0.1 mg/kg, mercury was < 0.01 mg/kg and cadmium ranged between 0.08 and 0.09 mg/kg. Aflatoxins B1, B2, G1, G2 and total aflatoxins analysed in the same batches were all below the limit of detections (LODs) (0.01 mg/kg) or < 0.03 mg/kg. 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

Two sets of stability results are described, each based on three batches of the product which were adjusted with maltodextrin to 1×10^{11} CFU/g and with dextrose to 2.5×10^{10} CFU/g, respectively. For both sets, samples were stored in sealed aluminium foil bags and stored at room temperature (not specified) for 1, 6, 12 and 18 months. No significant losses were observed in any of the assays.

[6] Technical dossier/Section II/Annex II.9.
[7] Technical dossier/Section II and Supplementary information November 2016/Annex II.5.
[8] Technical dossier/Section II/Annex II.2.
[9] Technical dossier/Section II/Annex II.1.
[10] This section has been amended following the confidentiality claims made by the applicant.
[11] Technical dossier/Section II/Annex II.4.

To test the stability in water, one batch of additive was adjusted to 10^{11} CFU/g with dextrose, diammonium phosphate and dipotassium phosphate, and three replicates of 5 g of this preparation were dissolved in 1 L water each, to give a count of 1×10^6 CFU/L. The solution was kept at room temperature (not specified) for 7 days, and samples were taken at different times for cell count. Counts of pediococci remained constant up to 3 days and decreased slightly (ca 20%) after 7 days.

3.1.4. Conditions of use

The additive is intended for use with all forages and for all animal species at a proposed minimum concentration of 1.0×10^8 CFU/kg fresh matter if applied alone, or 5.0×10^7 CFU/kg fresh matter if applied with other microorganisms. It can be applied dry or dispersed in water.

3.2. Safety

In the view of the FEEDAP Panel, the antibiotic resistance qualification has been met and the identity of the strain established as *Pediococcus parvulus*. Therefore, *P. parvulus* DSM 28875 is considered by EFSA to be suitable for the QPS approach to safety assessment and, consequently, is presumed safe for the target species, consumers of products from animals fed treated silage and the environment.

3.2.1. 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 to be 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. 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 used in the preparation of the final formulation do not introduce additional risks.

3.3. Efficacy

Five laboratory experiments were made using different forage samples. The duration of the experiments was 90 days. In all of the studies, forage was ensiled in 4.5-L minisilos fitted with air locks to vent gas. The ambient temperature during ensiling was 20 ± 2°C. The additive was dissolved in water and sprayed on the forage at an intended concentration of 5×10^7 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.[12] Four replicate silos were prepared for each experimental treatment (without or with the additive). The forages used were mixtures of grasses, legumes and herbs with different botanical composition and different dry matter (DM) and water-soluble carbohydrates (WSC) contents (see Table 1) to represent material easy to ensile (studies 1,[13] 2[14] and 3[15]), moderately difficult to ensile (study 4[16]) and difficult to ensile (study and 5[17]), as specified by Regulation (EC) No 429/2008.

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

Study	Test material	Dry matter content (%)	Water-soluble carbohydrate content (% fresh matter)
1	Grass/legume mixture (74:26)[(a)]	25.2	4.3
2	Grass/legume mixture (68:32)[(a)]	43.4	3.4
3	Grass/legume mixture (95:5)[(a)]	20.9	3.0
4	Grass/legume mixture (72:28)[(a)]	40.8	2.3

[12] Technical dossier/Section IV/Annexes IV.1 and IV.5.
[13] Technical dossier/Section IV/Annex IV.7.
[14] Technical dossier/Section IV/Annex IV.4.
[15] Technical dossier/Section IV/Annex IV.6.
[16] Technical dossier/Section IV/Annex IV.3.
[17] Technical dossier/Section IV/Annex IV.2.

Study	Test material	Dry matter content (%)	Water-soluble carbohydrate content (% fresh matter)
5	Grass/legume mixture (33:67)[(a)]	21.8	1.2

(a): Grass and legume percentages in the mixture, where the predominant legumes were red clover and lucerne, and the grasses were predominately timothy and meadow fescue.

Silos were opened at the end of the experiment and the contents were analysed by conventional methods to determine silage DM and WSC contents, pH, lactic and volatile fatty acid concentrations, ethanol, ammonia and total nitrogen. DM loss during ensiling was calculated and corrected for volatiles loss according to Weissbach and Strubelt (2008). Statistical evaluation of data was by a non-parametric test (Wilcoxon Kruskal–Wallis test) to compare treated versus control silos. Significance was declared at $p < 0.05$.

Table 2: Summary of the analysis of ensiled material recovered at the end of the ensiling period (90 days) with *Pediococcus parvulus* DSM 28875 at a dose of CFU/kg

Study	Application rate (CFU/kg forage)	Dry matter loss (%)	pH	Lactic acid (% dry matter)	Acetic acid (% dry matter)	Ammonia-N (% total N)
1	0	8.5	4.6	5.7	0.9	10.7
	5×10^7	2.5*	4.0*	10.6*	0.7*	6.9*
2	0	2.2	4.6	7.2	1.6	6.4
	5×10^7	2.0	4.4*	7.2	1.0*	6.6
3	0	12.1	5.2	0.9	2.5	23.5
	5×10^7	2.6*	4.0*	10.4*	0.9*	8.0*
4	0	1.7	4.8	4.5	1.1	7.3
	5×10^7	1.3*	4.3*	6.3*	0.6*	6.5*
5	0	3.9	4.6	7.8	3.6	8.8
	5×10^7	3.7	4.5*	8.6*	3.5	8.4

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

Addition of *P. parvulus* at 5×10^7 CFU/kg decreased pH and increased the concentration of lactic acid, but had no effect on DM losses or ammonia-N in silos with difficult to ensile material (Table 2, study 5). With the moderately difficult to ensile material (study 4) and two of the easy to ensile materials (studies 1 and 3), the additive decreased pH, increased lactic acid concentration and decreased both DM loss and ammonia-N (Table 2). However, with the other easy to ensile forage (study 2), the additive only decreased pH and acetic acid concentration, but did not affect the other parameters.

Considering the effects on ammonia-N as percentage of total N, indicating a better preservation of protein in silage, it can be concluded that the additive has the potential to improve the preservation of nutrients in silage prepared from easy and moderately difficult to ensile material.

4. Conclusions

As the identity of the strain *Pediococcus parvulus* DSM 28875 has been established and no antibiotic resistance of concern 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 or skin sensitisation of the additive. The additive should be considered as a potential respiratory sensitiser.

The addition of *Pediococcus parvulus* DSM 28875 at 5×10^7 CFU/kg forage has the potential to improve the production of silage from easy and moderately difficult to ensile forage species by reducing DM loss and enhancing protein preservation.

Documentation provided to EFSA

1) *Pediococcus parvulus* DSM 28875. Request for authorisation according to Regulation (EC) 1831/2003 Article 4(1). March 2016. Submitted by Microferm Ltd.

2) *Pediococcus parvulus* DSM 28875. Request for authorization according to Regulation (EC) 1831/2003 Article 4(1). Supplementary information November 2016. Submitted by Microferm Ltd.
3) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for *Pediococcus parvulus* DSM 28875.
4) Comments from Member States.

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. doi:10.2903/j.efsa.2007.587

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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. doi:10.2903/j.efsa.2012.2740

Weissbach F and Strubelt C, 2008. Correcting the dry matter content of grass silages as a substrate for biogas production. Landtechnik 63, 210–211.

Abbreviations

BIOHAZ	EFSA Panel on Biological Hazards
CFU	colony-forming unit
DM	dry matter
DSMZ	German Collection of Microorganisms and Cell Cultures
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
HACCP	hazard analysis and critical control points
LOD	limit of detection
MIC	minimum inhibitory concentration
MLS	multilocus sequence
PFGE	pulsed field gel electrophoresis
QPS	Qualified Presumption of Safety
RAPD	random amplification of polymorphic DNA
WSC	water-soluble carbohydrates

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 parvulus* DSM 28875

In the current application authorisation is sought under Article 4(1) for *Pediococcus parvulus* DSM 28875 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 non-genetically modified strain *Pediococcus parvulus* DSM 28875. The *feed additive* is to be marketed as a powder containing a minimum *Pediococcus parvulus* DSM 28875 concentration of 8×10^{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 5×10^{7} CFU/kg fresh *silage*.

For the identification of *Pediococcus parvulus* DSM 28875, the EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised standard methodology for microbial identification.

For enumeration of *Pediococcus parvulus* DSM 28875 in *feed additive*, the Applicant submitted the ring-trial validated spread plate method EN 15787 specifically designed for the analysis of Lactobacillus spp. The EURL identified instead the ring-trial validated spread plate method EN 15786:2009 dedicated to the "*enumeration of Pediococcus spp*.". Based on the performance characteristics available, the EURL recommends for official control the EN 15786:2009 method for the enumeration of *Pediococcus parvulus* DSM 28875 in the *feed additive per se*.

The Applicant did not provide any experimental method or data for the determination of *Pediococcus parvulus* DSM 28875 in *silage*. Since the unambiguous determination of the content of *Pediococcus parvulus* DSM 28875 initially added to silage is not achievable by analysis, the EURL cannot evaluate or 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) is not considered necessary.