SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of *Pediococcus acidilactici* (CNCM I-3237, CNCM MA 18/5M—DSM 11673) and *Pediococcus pentosaceus* (DSM 23376, NCIMB 12455, NCIMB 30237 and NCIMB 30168) as silage additives for all species[1]

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Two strains of *Pediococcus acidilactici* and four strains of *P. pentosaceous* are each intended to improve ensiling at proposed doses ranging from 1.4×10^7 to 1.0×10^8 colony-forming units (CFUs)/kg fresh material. Both bacterial species are considered by EFSA to be suitable for the Qualified Presumption of Safety approach to safety assessment. As the identity of all strains was clearly established and as no antibiotic resistance of concern was detected, the use of these strains in silage production is presumed safe for livestock species, consumers of products from animals and the environment. The material safety data sheet proposed indicates that preparations containing the strains may cause irritation on contact with skin or eyes. In addition, given the dusting potential and proteinaceous nature of the active agents, the Panel considers it prudent to treat all six additives as skin and respiratory sensitisers. Studies with laboratory-scale silos are described for each strain, each lasting at least 90 days, made using forage samples representing materials that are easy to ensile, moderately difficult to ensile and difficult to ensile. One strain of *P. acidilactici* and three strains of *P. pentosaceus* were shown to have the potential to improve the production of silage from forage species that were easy, moderately difficult and difficult to ensile by reducing the pH and increasing the preservation of dry matter and/or protein, when used at the doses proposed. Another strain of *P. pentosaceus* appeared to favourably affect the ensiling process by reducing the pH, but only when used in combination with a specific strain of *Lactobacillus plantarum*. However, the consequences of a more rapid reduction in pH for the preservation of nutrients were not shown. Data for the remaining strain of *P. acidilactici* were partly contradictory and inconsistent and no conclusions on efficacy could be drawn.

KEY WORDS

Technological additive, silage additive, *Pediococcus acidilactici*, *Pediococcus pentosaceus*, QPS, safety, efficacy

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[1] On request from the European Commission, Question No EFSA-Q-2011-00940, adopted on 23 May 2012.

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SUMMARY

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 the safety for the target animals, consumer, user and the environment and on the efficacy of products based either on one of the two specific strains of *Pediococcus acidilactici* or on one of the four specific strains of *Pediococcus pentosaceus* when used as a technological additive intended to improve the ensiling process at proposed doses ranging from 1.4×10^7 to 1.0×10^8 colony-forming units (CFUs)/kg fresh material.

The bacterial species *P. acidilactici* and *P. pentosaceus* are considered by EFSA to be suitable for the Qualified Presumption of Safety (QPS) approach to safety assessment. Therefore, strains belonging to these species do not require any specific demonstration of safety, other than confirming the absence of any determinants of resistance to antibiotics of human and veterinary clinical significance and the safety for the user. As the identity of all six strains was clearly established and as no antibiotic resistance of concern was detected, the use of the six strains in the production of silage is presumed safe for livestock species, consumers of products from animals fed the treated silage and the environment.

No data are available on skin or eye irritation for any of the strains in any formulation. However, the generic material safety data sheet proposed for the six strains indicate that preparations containing the strains may cause irritation on prolonged contact with skin and eye irritation upon direct contact. Although users at the farm level are exposed to the additive only for a short period of time when preparing the aqueous suspension, the FEEDAP Panel considers it prudent, given the proteinaceous nature of the active agents, to treat all six additives as skin and respiratory sensitisers.

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 listed several cryoprotectants and carriers that would allow multiple formulations of the additives to be produced 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 concerns. For the products described in this application, all excipients listed as likely to be used are food grade or equivalent, and their use in the additive would not introduce any risk additional to their conventional use.

Studies with laboratory-scale silos are described for each strain, each lasting at least 90 days, made using forage samples of differing water-soluble carbohydrate content and representing material from the range easy to ensile, moderately difficult to ensile and difficult to ensile. In each case, silos containing treated forage were compared with identical silos containing the same untreated forage. Replicate silos were opened at the end of the experiment and the contents were analysed for dry matter content, pH, lactic and volatile fatty acids concentration, ethanol, ammonia and total nitrogen. One strain of *P. acidilactici* and three strains of *P. pentosaceus* were shown to have the potential to improve the production of silage from forage species that were easy, moderately difficult and difficult to ensile by reducing the pH and increasing the preservation of dry matter and/or protein, when used at the doses proposed. Another strain of *P. pentosaceus* appeared to favourably affect the ensiling process by reducing the pH, but only when used in combination with a specific strain of *Lactobacillus plantarum*. However, the consequences of a more rapid reduction in pH for the preservation of nutrients were not shown. Data for the remaining strain of *P. acidilactici* were partly contradictory and inconsistent and no conclusions on efficacy could be drawn.

TABLE OF CONTENTS

BACKGROUND

Regulation (EC) No 1831/2003[4] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular Article 10(2)/(7) of that Regulation specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, within a maximum of seven years after the entry into force of this Regulation.

The European Commission received a request from the company SILAC-EEIG-Silage Additives[5] for re-evaluation of the products *Pediococcus acidilactici* CNCM I-3237, *Pediococcus acidilactici* CNCM MA 18/5M – DSM 11673, *Pediococcus pentosaceus* DSM 23376, *Pediococcus pentosaceus* NCIMB 12455, *Pediococcus pentosaceus* NCIMB 30237 and *Pediococcus pentosaceus* NCIMB 30168 to be used as feed additives for all animal species (category: technological additive; functional group: silage additive) under the conditions mentioned in Table 1.

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 10(2)/(7) (re-evaluation of an authorised feed additive). EFSA received directly from the applicant the technical dossier in support of this application.[6] According to Article 8 of that Regulation, 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. The particulars and documents in support of the application were considered valid by EFSA as of 31 August 2011.

These products were included in the Euorpean Union Register of Feed Additives following the provisions of Article 10(1) of Regulation (EC) No 1831/2003.

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall 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 the efficacy of the products *Pediococcus acidilactici* CNCM I-3237, *Pediococcus acidilactici* CNCM MA 18/5M – DSM 11673, *Pediococcus pentosaceus* DSM 23376, *Pediococcus pentosaceus* NCIMB 12455, *Pediococcus pentosaceus* NCIMB 30237 and *Pediococcus pentosaceus* NCIMB 30168, when used under the conditions described in Table 1.

[4] 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.

[5] SILAC-EEIG-Silage Additives (Consortium involving the Companies Alltech, Lallemand SAS, Agri-King Ltd, Micron Bio-Systems, and Volac International Ltd), Avenue Louise, 120-Box 13, 1050, Brussels, Belgium.
EFSA Dossier reference: FAD-2010-0127.

Table 1: Description and conditions of use of the additive as proposed by the applicant

Additive	*Pediococcus acidilactici* (CNCM I-3237, CNCM MA 18/5M – DSM 11673) and *Pediococcus pentosaceus* (DSM 23376, NCIMB 12455, NCIMB 30237 and NCIMB 30168)			
Registration number/EC No/No	-			
Category(ies) of additive	Technological			
Functional group(s) of additive	Silage additive			
Description				
Composition, description	Chemical formula	Purity criteria	Method of analysis	
Pediococcus acidilactici CNCM I-3237 with a minimum content of $2x10^{10}$ CFU/g. *Pediococcus acidilactici* CNCM MA 18/5M – DSM 11673, *Pediococcus pentosaceus* NCIMB 12455, each with a minimum content of $3x10^{9}$ CFU/g. *Pediococcus pentosaceus* DSM 23376 with a minimum content of $1x10^{11}$ CFU/g. *Pediococcus pentosaceus* NCIMB 30237 with a minimum content of $2x10^{11}$ CFU/g. *Pediococcus pentosaceus* NCIMB 30168 with a minimum content of $5x10^{10}$ CFU/g.		Significant impurities: - Coliforms: <1000 CFU/g - Yeast and molds: <1000 CFU/g Relevant impurities: - *E. coli*: <10 CFU/g - *Salmonella*: absence in 25g - Aflatoxin B1: <1µg/kg	Enumeration method EN 15786:2009 Identification method (genetic): PFGE	
Trade name	Not applicable			
Name of the holder of authorisation	Not applicable			
Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period
		CFU/kg of complete feedingstuffs		
All species	n.a.	1 x 10^{8} (easy, moderate	n.a.	n.a.

and animal categories		and difficult forage) for *P. pentosaceus* NCIMB 30168 and 1 x 10^8 (easy, moderate forages) for *P. pentosaceus* DSM 23376 3 x 10^7 (easy, moderate and difficult forage) for *P. pentosaceus* NCIMB 12455 and for *P. acidilactici* CNCM MA 18/5M - DSM 11673 1.4 x 10^7 (easy, moderate and difficult forage) for *P. acidilactici* CNCM I-3237 8 x 10^7 (easy, moderate and difficult forage) for *P. pentosaceus* NCIMB 30237		

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use (if appropriate)	In the direction for use indicate the storage temperature, and storage life.
Specific conditions or restrictions for handling (if appropriate)	For safety: eye protection and gloves shall be used during handling
Post-market monitoring (if appropriate)	n.a.
Specific conditions for use in complementary feedingstuffs (if appropriate)	n.a.

Maximum Residue Limit (MRL) (if appropriate)			
Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues
n.a.	n.a.	n.a.	n.a.

ASSESSMENT

1. Introduction

Six genera of lactic acid-producing bacteria are commonly associated with forage species and collectively contribute to the natural ensiling process. The present application concerns six different strains of two single species of one of these six genera, *Pediococcus acidilactici* and *Pediococcus pentosaceus*. Each strain is intended to be added to forages to promote ensiling (technological additive, functional group: silage additive) for eventual use of the silage in any animal species.

One of the strains, *P. acidilactici* CNCM MA 18/5M, is already authorised as a zootechnical feed additive for salmonids, shrimp and chickens and pigs for fattening (E 1712).

Both species *P. acidilactici* and *P. pentosaceus* are considered by EFSA to be suitable for the Qualified Presumption of Safety (QPS) approach to safety assessment (EFSA, 2007, 2011). This approach requires the identity of the strain to be conclusively established and evidence that the strains do not show acquired resistance to antibiotics of human and veterinary importance.

2. Characterisation

2.1. Identity and properties of the active agent

The six strains of the two species included in this application are listed in Table 2, together with their accession numbers in internationally recognised culture collections. Each strain has been given a reference letter which, for convenience, will be used throughout this opinion. Accession numbers for which a copy of the certificate of deposition is provided are shown in bold.[7]

Table 2: The strains of *P. acidilactici* and *P. pentosaceus* and their accession numbers

Ref letter	Accession number(s)
A	*Pediococcus acidilactici* ATCC 8042—**CNCM I-3237**
B	*Pediococcus acidilactici* **CNCM MA 18/5—DSM 11673**
C	*Pediococcus pentosaceus* NCIMB 30171—**DSM 23376**
D	*Pediococcus pentosaceus* **NCIMB 12455**
E	*Pediococcus pentosaceus* **NCIMB 30237**
F	*Pediococcus pentosaceus* **NCIMB 30168**

ATCC,American Type Culture Collection; CNCM, Collection Nationale de Culture de Microorganismes; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen; NCIMB, National Collection of Industrial and Marine Bacteria.

All strains were isolated from silage with the exception of strains A and C (unspecified plant material). None of the strains have been genetically modified.

Taxonomic identification of all strains was established by partial 16S rRNA gene sequence analysis and phenotypical tests. Strain-specific identification and genetic stability analysis are based on the use of pulsed-field gel electrophoresis and, in the case of strain C, a *recA* polymerase chain reaction test. Using this method the master cultures are routinely compared with working cultures used to inoculate fermentation batches. No differences in the resultant patterns have been observed to date.

Each strain was tested for antibiotic susceptibility using twofold broth dilutions.[8] The battery of antibiotics tested generally included all of those recommended by EFSA (EFSA, 2008) but excluding vancomycin, which is not required for these species. As all minimum inhibitory concentration values

[7] Technical dossier/ Section II/Annexes 2-2-10 to 15.
[8] Technical dossier/Section II/Annexes 2-2-17 to 22.

for the six strains were equal or lower than the corresponding cut-off values defined by the FEEDAP Panel, no further investigation is required.

2.2. Production and characteristics of the additive

The active agents are grown in sterilised media typical of those used for lactic acid bacteria. Typical ingredients are listed and material safety data sheets provided and all are of food grade and do not give rise to safety concerns. Cells then are separated from the growth medium by centrifugation or microfiltration, cryoprotectants (ascorbic acid and dipotassium phosphate anhydrous) are added and the cell mix is freeze-dried and ground. The ground powder is then blended with sufficient carrier to meet the minimum specified concentration for each additive.

The composition, minimum specified content and analysed content of the active agent is shown for each additive in Table 3. Maximum values for the spent medium and cyroprotectants appear to be expressed as a percentage of the product after freeze-drying but prior to the adjustment of cell numbers with the carrier.

Table 3: Composition of the six additives and the minimum guaranteed content of the active agent

Additive	Minimum guaranteed cell count (CFUs/g)	Fermentation medium (%)	Cryoprotectants (%)
A	5×10^9	3.3	50.6
B	3×10^9	10.0	68.0
C	2×10^{10}	25.6	41.6
D	3×10^9	9.2	65.6
E	2.6×10^{10}	14.0	38.0
F	1×10^{10}	10.0	53.7

The additives are routinely monitored for microbial contamination. Limits are set for *Enterobacteriaceae* and yeasts and filamentous fungi (< 10^3 CFUs/g additive), *Escherichia coli* (< 10 CFUs/g additive) and *Salmonella* (absence in 25 g additive) and aflatoxin B1 (< 1.0 µg/kg additive). All data (three to four batches of each additive) confirmed compliance with these limits for yeasts and fungi, *Enterobacteriaceae*, *Salmonella* and *E. coli.* Given the nature of the fermentation medium and the food-grade excipients, the probability of contamination with heavy metals or mycotoxins is low. One to four batches of each additive were analysed for aflatoxin B1 and comply with the specification.[9]

One to four batches of the additives (excipients unknown) were examined for particle size distribution by laser diffraction and for dusting potential using a Heubach dustmeter. Two (A and F) were measured as an intermediate in the production process and the others (B, C, D and E) as final formulations. The mean particle size of the different test items was 210, 140, 82, 96, 130 and 46 µm in A, B, C, D, E and F, respectively. The fraction of particles with diameters below 50 µm[10] amounted to 4.4 % in A, about 30 % in B, C, D and E and 10 % in F. Dusting potential for the six strains was provided in a variety of formulations including silage premixtures. Consequently, the data can be taken as indicative only. Values for preparations containing strains A to F were approximately 1.2, 0.7, 18.3, 0.7, 19.2 and 3.2 g/m^3, respectively.

[9] Technical dossier/Section II/Annex 2-1-23 to 28.
[10] Technical dossier/Section II/Annex 2-1-29 to 35.

2.3. Shelf-life and stability in water

Shelf-life was shown for strains A, B, C and D to be at least 12 months at a temperature of 20 ºC when stored in sealed moisture-tight containers.[11] Evidence of stability for six months at ambient temperature was provided for strains E and F.

The short-term stability in water was determined individually for all of the strains under application.[12] Three batches of each strain (except for E, one only) were separately diluted into water in concentrations mimicking the proposed application rate, stored under ambient conditions (20 ºC) and bacterial counts made after 24 and 48 hours. All strains showed little or no losses after 24 hours. Some loss of viability was recorded for some of the strains after 48 hours but this did not exceed 0.5 log count and was compensated for by the overage practised in formulation.

2.4. Conditions of use

The additives are intended for use with forages at a proposed minimum dose shown in Table 4 and applied directly to silage (granular application) or as an aqueous suspension.

Table 4: Application and recommended dose

Strain	Type of forage	Recommended dose (CFUs/kg fresh silage)
A: *P. acidilactici* CNCM I-3237	All forages	1.4×10^7
B: *P. acidilactici* CNCM MA 18/5M – DSM 11673	All forages	3×10^7
C: *P. pentosaceus* DSM 23376	All forages	1×10^8
D: *P. pentosaceus* NCIMB 12455	All forages	3×10^7
E: *P. pentosaceus* NCIMB 30237	All forages	8×10^7
F : *P. pentosaceus* NCIMB 30168	All forages	1×10^8

2.5. Evaluation of the analytical methods by the European Union Reference Laboratory (EURL)

EFSA has verified the EURL report as it relates to the methods used for the control of the active agents in animal feed. The executive summary of the EURL report can be found in the Appendix.

3. Safety

In the view of the FEEDAP Panel, the antibiotic resistance qualification has been met and the identity of the six strains established. Consequently, *P. acidilactici* and *P. pentosaceus* are suitable for the QPS approach to safety assessment, no further assessment of safety for the target species, consumers of products from animals fed treated silage or the environment is required, and the six strains are presumed safe for the target species, consumers of products from animals fed treated silage and the environment.

No data are available on skin or eye irritation for any of the strains in any formulation. However, the generic material safety data sheets proposed for the six strains indicate that preparations containing the strains may cause irritation on prolonged contact with skin and eyes.

Exposure via a respiratory route was not directly considered. However, given that a significant proportion (10–30 %) of five of the six products consists of particles with diameters below 50 µm, there is a risk of respiratory sensitisation. Although users at the farm level are exposed to the additive only for a short period of time when preparing the aqueous suspension, given the lack of specific

[11] Technical dossier/Supplemenatry information March 12/Annexes Qi.
[12] Technical dossier/Supplemenatry information March 12/Annexes Qii.

information and the proteinaceous nature of the additive, the active agents should be considered to have the potential to be skin/respiratory sensitisers.

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 listed several cryoprotectants and carriers that would allow multiple formulations of the additive to be produced, 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 concerns. For these products, all excipients used are food grade, and their inclusion in the additive would not introduce any risk additional to that posed by their conventional use.

4. Efficacy

A total of 20 laboratory studies are described, each of at least 90 days' duration. The studies were made using mini-silos with a capacity of 0.6–2.5 L, except in experiments 7–9, in which 113-L barrels were used. The number of replicates varied between three and seven (see Table 5). Replicate silos were sprayed with the additive at the recommended dose. Forage in the control silos was sprayed with an equal volume of water excluding the additive.

In the case of strain E, all studies were made with a commercial silage additive containing the strain of *P. pentosaceus* under application and a strain of *Lactobacillus plantarum* in a ratio of 8:2 by bacterial count. The applicant states that the *L. plantarum* strain used is the subject of a separate application (*L. plantarum* NCIMB 30238) and thus safety will be assessed and reported in another opinion (EFSA, 2012). Consequently, conclusions can be drawn only subject to the successful outcome of that safety assessment.

Bacterial counts were confirmed by analysis of the applied solutions, except in additives A and E. The experiments were conducted at approximately 20 ± 2°C with a range of forages of differing water-soluble carbohydrate content and representing material that are easily, moderately difficult and difficult to ensile, as defined in Regulation (EC) 429/2008 (see Table 5).

Table 5: Characteristics of the grass samples used in the three ensiling experiments

Study	Strain	Test material	Dry matter content (%)	Water-soluble carbohydrate content (% fresh matter)
1[13]	A	Perennial rye grass	27.2	4.8
2[14]		Red clover	15.0	1.5
3[15]		Lucerne	18.7	1.5
4[16]	B	Italian rye grass	36.7	4.1
5[17]		Permanent grass	32.1	2.5
6[18]		Lucerne	34.5	1.4
7[19]	C	Lucerne	57.4	6.4
8[20]		Rye grass	12.2	1.8
9[21]		Grass	17.0	0.2
10[22]	D	Italian rye grass	36.7	4.1
11[23]		Permanent grass	32.1	2.5
12[24]		Lucerne	34.5	1.4
13[25]	E	Maize	27.6	3.2
14[26]		Italian rye grass	22.1	2.1
15[27]		Lucerne	31.7	2.4
16[28]		Lucerne	30.5	1.1
17[29]		Grass/lucerne	26.4	0.5
18[30]	F	Tall fescue	32.8	5.8
19[31]		Red clover	25.0	1.5
20[32]		Lucerne	24.0	1.1

Data were examined by the Student–Newman–Keuls test after confirming the normal distribution of the dataset using one-way analysis of variance (ANOVA) (experiments 1–3), ANOVA with a post-hoc Tukey test (based on a total of seven treatments) or the Wilcoxon non-parametric procedure (experiments 4–6 and 10–12), two-way ANOVA with a least significant difference test (experiments 7-9), the generalised linear model procedure with a post-hoc Tukey test (experiments 13-17) and the Wilcoxon–Kruskal–Wallis test (experiments 18-20).

[13] Technical dossier/Section IV/Annex 4.1.
[14] Technical dossier/Section IV/Annex 4.1.
[15] Technical dossier/Section IV/Annex 4.1.
[16] Technical dossier/Section IV/Annex 4.2.
[17] Technical dossier/Section IV/Annex 4.2.
[18] Technical dossier/Section IV/Annex 4.2.
[19] Supplementary information Section IV/Annex_Qiv.
[20] Supplementary information Section IV/Annex_Qiv.
[21] Supplementary information Section IV/Annex_Qiv.
[22] Technical dossier/Section IV/Annex 4.4.
[23] Technical dossier/Section IV/Annex 4.4.
[24] Technical dossier/Section IV/Annex 4.4.
[25] Technical dossier/Section IV/Annex 4.5.
[26] Technical dossier/Section IV/Annex 4.5.
[27] Technical dossier/Section IV/Annex 4.5.
[28] Technical dossier/Section IV/Annex 4.5.
[29] Technical dossier/Section IV/Annex 4.5.
[30] Technical dossier/Section IV/Annex 4.6.
[31] Technical dossier/Section IV/Annex 4.6.
[32] Technical dossier/Section IV/Annex 4.6.

Table 6: Summary of the analysis of ensiled material recovered at the end of the experiments

Study (no of replicates)	Dose (CFUs/kg forage)	Dry matter loss (%)	pH	Lactic acid (% ensiled material)	Acetic acid (% ensiled material)	Ethanol (% ensiled material)	$N\text{-}NH_3$ (% total N)
Strain A							
1 (5)	0 1×10^7	4.1 10.4*	3.6 3.6	2.3 2.2	0.5 0.2*	0.0 0.2	9.9 9.9
2 (5)	0 3×10^7	17.5 9.3*	3.8 3.8	1.5 1.6	0.3 0.2	0.1 0.1	13.5 13.4
3 (5)	0 1×10^7	17.1 10.8*	6.2 5.1*	0.0 1.0*	0.3 0.6	0.1 0.2	23.1 22.6
Strain B							
4 (3)	0 3×10^7	1.6 1.2*	4.2 4.0*	1.3 2.1*	1.2 0.8*	0.0 0.0	5.7 5.5
5 (3)	0 1×10^7	1.1 0.7*	4.4 4.1*	1.8 2.5*	0.5 0.4*	0.0 0.0	7.9 6.4*
6 (3)	0 3×10^7	1.0 0.9*	4.4 4.3*	2.3 2.3	0.8 0.7	0.7 0.7	9.2 8.1*
Strain C							
7 (3)	0 1×10^8	0.6 0.3*	5.5 5.0*	2.0 7.4*	0.5 0.2*	0.8 0.6*	7.4 6.6*
8 (4)	0 1×10^8	6.3 6.2	4.1 4.1	1.5 1.6	0.6 0.2*	0.1 0.1	12.5 9.8*
9 (3)	0 1×10^8	1.0 0.5*	4.2 4.1*	1.1 1.5*	1.2 0.4*	0.2 0.1*	17.9 11.8*
Strain D							
10 (5)	0 8×10^7	1.6 1.2*	4.2 3.9*	1.3 1.9*	1.2 0.6*	– –	5.7 5.3
11 (5)	0 1×10^8	1.1 0.5*	4.4 4.1*	2.1 2.3	0.5 0.5	– –	7.9 5.2*
12 (5)	0 1×10^8	1.0 0.9*	4.3 4.3	2.3 2.4	0.8 0.7*	1.1 1.0	9.2 9.1
Strain E**							
13 (7)	0 3×10^7	- -	3.6 3.6	2.4 2.7*	0.6 0.6	– –	– –
14 (3)	0 8×10^7	- -	4.1 4.0*	2.0 2.3*	– –	– –	– –
15 (3)	0 8×10^7	- -	5.2 4.1*	1.3 2.4*	0.6 0.5	0.3 0.2*	– –
16 (3)	0 1×10^8	- -	4.4 4.1*	2.4 2.8*	0.6 0.4*	– –	– –
17 (3)	0 3×10^7	- -	4.1 3.9*	2.2 2.5*	0.5 0.5	– –	– –

Strain F							
18	0	14.3	4.6	1.0	0.1	0.4	19.9
(4)	8×10^7	8.7*	3.9*	2.0*	0.1	0.8*	16.4*
19	0	20.0	3.8	1.5	0.3	0.1	14.0
(4)	8×10^7	15.4	3.6*	1.7*	0.2*	0.1	10.8*
20	0	11.5	5.8	0.6	0.9	0.2	35.4
(4)	1×10^8	5.9*	4.8*	1.9*	0.7*	0.1*	21.5*

*Significantly different from the control value at $P < 0.05$.
**When used in combination with *Lactobacillus plantarum* NCIMB 30238.

The results of the experiments indicated that strains B, C, D and F have the potential to improve the production of silage from forage species that are easy, moderately difficult and difficult to ensile by reducing the pH and increasing the preservation of dry matter and/or protein.

Strain E showed the potential to improve the production of silage from a similar range of forage species by reducing the pH, but only when used in combination with *L. plantarum* NCIMB 30238 in a ratio of 8:2 by bacterial count. However, the consequences of a more rapid reduction in pH for the preservation of nutrients were not shown.

Data for strain A were partly contradictory and inconsistent. Although some beneficial effects were shown in two studies, the opposite effect was shown in the third. Consequently, no conclusions on efficacy can be drawn.

CONCLUSIONS

As the identity of all of the strains of *P. acidilactici* and *P. pentosaceus* under application has been established and no antibiotic resistance of concern detected, following the QPS approach the use of these strains in the production of silage is presumed safe for target species, consumers of products from animals fed treated silage and the environment.

The generic material safety data sheet proposed for the six strains indicates that preparations containing the strains may cause irritation on prolonged contact with skin, and eye irritation upon direct contact. A significant fraction of these products with particles that are potentially inhalable means that exposure via the respiratory route is a hazard. Although users at the farm level are exposed to the additive for only a short period of time when preparing the aqueous suspension, the FEEDAP Panel considers it prudent, given the proteinaceous nature of the active agents, to treat all six additives as skin and respiratory sensitisers.

The additives containing *P. acidilactici* CNCM MA 18/5M—DSM 11673 (strain B), and the *P. pentosaceus* strains DSM 23376 (strain C), NCIMB 12455 (strain D) and NCIMB 30168 (strain F) have the potential to improve the production of silage from forage species that are easy, moderately difficult and difficult to ensile by reducing the pH and increasing the preservation of dry matter and/or protein, when used at the doses proposed.

P. pentosaceus NCIMB 30237 (strain E) appeared to favourably affect the ensiling process by reducing the pH, but only when used in combination with *L. plantarum* NCIMB 30238 in a ratio of 8:2 by bacterial count. However, the consequences of a more rapid reduction in pH for the preservation of nutrients were not shown.

Data for strain *P. acidilactici* CNCM I-3237 (strain A) were partly contradictory and inconsistent and no conclusions on efficacy could be drawn.

REMARK

The minimum concentrations listed for the different additives in Table 1 do not correspond to the values given in the dossier.

DOCUMENTATION PROVIDED TO EFSA

1. *Pediococcus acidilactici* (2 strains) and *Pediococcus pentosaceus* (4 strains). September 2010. Submitted by SILAC-EEIG-Silage Additives.

2. *Pediococcus acidilactici* (2 strains) and *Pediococcus pentosaceus* (4 strains). Supplementary information. March 2012. Submitted by SILAC-EEIG-Silage Additives.

3. Evaluation report of the European Union Reference Laboratory for Feed Additives on the methods of analysis for *Pediococcus acidilactici* (2 strains) and *Pediococcus pentosaceus* (4 strains).

4. Comments from Member States received through the ScienceNet.

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. The EFSA Journal, 587, 1–16.

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EFSA Panel on Biological Hazards (BIOHAZ), 2011. Scientific opinion on the maintenance of the list of QPS biological agents intentionally added to food and feed (2011 update). EFSA Journal, 9(12):2497.

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APPENDIX

Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Methods of Analysis for *Pediococcus acidilactici* and *Pediococcus pentosaceus* for all animal species[33]

This report is on the evaluation of feed additives "*micro-organisms used as silage agents*", which is related to the application of ten micro-organisms for which authorisation is sought under Article 10(7). Authorisation is sought for all the above mentioned micro-organisms under category/functional group 1(k), "technological additives/silage additives", according to Annex I of Regulation (EC) No 1831/2003. The list of *micro-organisms* of interest and the minimum activities in the feed additives and in silage, as sought in the authorisation, are presented in Table 1.[34] The intended use of the current applications is for all animal species.

For identification and characterisation of all ten micro-organisms of concern (i.e. *Lactobacilli* and *Pediococci*) the EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised standard methodology for microbial identification.

The EURL recommends for enumeration in the feed additives the following ring trial validated methods:

- Spread plate method using MRS agar (EN 15787) for *Lactobacilli*; and
- Spread plate method using MRS agar (EN 15786) for *Pediococci.*

None of the Applicants provided experimental data for the determination of *micro-organisms* in *silage*. Furthermore, the unambiguous determination of the content of *micro-organisms* added to *silage* is not achievable by analysis. Therefore the EURL cannot evaluate nor recommend any method for official control to determine any of the ten micro-organisms of concern 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.

[33] The EURL produced a combined report for the *L. lactis, L. plantarum, L. buchneri, L. paracasei, L. rhamnosus, L. salivarius, L. casei L. brevis, L. pentosus, P. acidilactici, P. pentosaceus, Bacillus, Saccharomyces cerevisiae and Lactococcus lactis.*

[34] Full list provided in EURL evaluation report, available on the EURL website: http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2010-0127+0252+0259+0280.pdf