European Food Safety Authority

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SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of *Enterococcus faecium* (NCIMB 10415, DSM 22502, ATCC 53519 and ATCC 55593) as silage additives for all animal species[1]

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

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ABSTRACT

Four strains of *Enterococcus faecium* are each intended to improve the ensiling process at doses ranging from 5×10^6 to 1.0×10^8 colony-forming units (CFU)/kg forage. The strains do not contain marker genes typical of hospital-associated isolates responsible for clinical infections and are susceptible to clinically relevant antibiotics. Therefore, the use of these strains as silage additives is safe for consumers of animal products. It is not expected that the use of *E. faecium* at the doses proposed would substantially increase the exposure of animals given the silage. Therefore, the use of these strains in the preparation of silage is safe for the target animals. In the absence of evidence, these additives should be regarded as skin and eye irritants and potential skin sensitisers. Given the proteinaceous nature of the active agents, the Panel considers it prudent to treat these additives as respiratory sensitisers. Given the high dusting potential of most of the preparations tested, there is a need to take measures to minimise inhalation exposure of workers. The use of these strains as silage additives is considered safe for the environment. The results of efficacy studies showed that two of the *E. faecium* strains have the potential to improve the production of silage from easy, moderately difficult and difficult to ensile forage materials at a minimum dose of 1×10^8 CFU/kg fresh materials. Given the magnitude of the responses recorded and the absence of any substantive evidence of nutrient preservation, the data for the other two *E. faecium* strains, taken overall, provide little evidence of a benefit when used in the production of silage.

KEY WORDS

technological additive, silage additive, *Enterococcus faecium*, safety, efficacy

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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 environment of four strains of *E. faecium*, and their efficacy, when used as technological additives intended to improve the ensiling process at a proposed dose range of 5×10^6 to 1.0×10^8 CFU/kg forage.

None of the four *E. faecium* strains was shown to contain marker genes typical of hospital-associated isolates responsible for clinical infections, and all were susceptible to clinically relevant antibiotics. In addition, no other sources of concern have been identified in the additives. Consequently, the FEEDAP Panel considers the use of these *E. faecium* strains as silage additives safe for consumers of animal products.

It is not expected that the use of *E. faecium* at the doses proposed would substantially increase the exposure of animals given silage as part of their rations. Therefore, the FEEDAP Panel considers that use of these strains in the preparation of silage is safe for the target animals.

In the absence of evidence, these additives should be regarded as skin and eye irritants and potential skin sensitisers. Given the proteinaceous nature of the active agents, the FEEDAP Panel considers it prudent to treat these additives as respiratory sensitisers. Given the high dusting potential of most of the preparations tested, there is a need to take measures to minimise inhalation exposure of workers.

The use of these strains as silage additives is considered safe for the environment.

Studies with laboratory-scale silos are described, in which samples of forage of differing water-soluble carbohydrate content were used. Replicate silos containing treated forage were compared with identical silos containing the same, but untreated, forage. Two of the *E. faecium* strains have the potential to improve the production of silage from easy, moderately difficult and difficult to ensile forage materials at a minimum dose of 1×10^8 CFU/kg fresh materials. Given the magnitude of the responses recorded and the absence of any substantive evidence of nutrient preservation, the data for the other two *E. faecium* strains, taken overall, showed little evidence of a benefit when used in the production of silage.

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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 FEFANA Asbl[5] for re-evaluation of the products *Enterococcus faecium* (NCIMB 10415, DSM 22502, ATCC 53519 and ATCC 55593), to be used as feed additives for all animal species (category: technological additive; functional group: silage additive) under the conditions mentioned in Table 1.[6]

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.[7] 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 1 February 2012.

These products were included in the European Union Register of Feed Additives following the provisions of Article 10(1) of Regulation (EC) No 1831/2003. A product based on *E. faecium* NCIMB 10415 (Cylactin) is currently authorised as a zootechnical additive for chickens and pigs for fattening,[8] piglets,[9] sows,[10] calves,[11] and cats and dogs.[12] The same strain is also authorised under a different trade name (Oralin) for calves,[13] piglets,[14] chickens for fattening,[15] turkeys for fattening and dogs.[16] A product based on *E. faecium* DSM 22502 (Lactiferm) is currently authorised as a zootechnical additive

[4] Regulation (EC) No 1831/2003 of the European parliament and the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.

[5] On 13/03/2013, EFSA was informed by the applicant that SILAC EEIG was liquidated on 19/12/2012 and their rights as applicant were transferred to FEFANA asbl (EU Association of Specialty Feed Ingredients and their Mixtures, representing notably the following companies: Agri-King Ltd., Christian Hansen A/S and Pioneer Hi-Breed Inc.). Avenue Louise, 130A, Box 1, 1050 Brussels, Belgium.

[6] In the course of the assessment the applicant has required the withdrawal of the request for authorisation of the strain *E. faecium* DSM 3530.

[7] EFSA Dossier reference: FAD-2010-0135.

[8] Commission Implementing Regulation (EU) No 361/2011 of 13 April 2011 concerning the authorisation of *Enterococcus faecium* NCIMB 10415 as a feed additive for chickens for fattening (holder of authorisation DSM Nutritional products Ltd represented by DSM Nutritional Products Sp. z o.o) and amending Regulation (EC) No 943/2005. OJ L 100, 14.4.2011, p. 22.

[9] Commission Regulation (EC) No 252/2006 of 14 February 2006 concerning the permanent authorisations of certain additives in feedingstuffs and the provisional authorisations of new uses of certain additives already authorised in feedingstuffs. OJ L 44, 15.2.2006, p. 3.

[10] Commission Regulation (EC) No 1200/2005 of 26 July 2005 concerning the permanent authorisation of certain additives in feedingstuffs and the provisional authorisation of a new use of an additive already authorised in feedingstuffs. OJ L 195, 27.7.2005, p. 6.

[11] Commission Regulation (EC) No 1288/2004 of 14 July 2004 concerning the permanent authorisation of certain additives and the provisional authorisation of a new use of an additive already authorised in feedingstuffs. OJ L 243, 15.7.2004, p. 10.

[12] Commission Regulation (EC) No 102/2009 of 3 February 2009 concerning the permanent authorisation of an additive in feedingstuffs. OJ L 34, 4.2.2009, p. 8.

[13] Commission Regulation (EC) No 255/2005 of 15 February 2005 concerning the permanent authorisations of certain additives in feedingstuffs. OJ L 45, 16.2.2005, p. 3.

[14] Commission Regulation (EC) No 1200/2005 of 26 July 2005 concerning the permanent authorisation of certain additives in feedingstuffs and the provisional authorisation of a new use of an additive already authorised in feedingstuffs. OJ L 195, 27.7.2005, p. 6.

[15] Commission Regulation (EC) No 1259/2004 of 8 July 2004 concerning the permanent authorisations of certain additives in feedingstuffs. OJ L 239, 9.7.2004, p. 8.

[16] Commission Regulation (EC) No 1520/2007 of 19 December 2007 concerning the permanent authorisations of certain additives in feedingstuffs. OJ L 355, 20.12.2007, p. 17.

for use in diets for piglets and calves.[17] A product based on the mixture of strains ATCC 53519 and 55593 (Probios PDFM) is authorised as a zootechnical additive for chickens for fattening.[18]

The Scientific Committee on Animal Nutrition issued on the safety for the target animals, consumers, users and environment of a product based on *E. faecium* NCIMB 10415 when used as a feed additive for chickens for fattening, piglets, pigs for fattening and calves (EC, 1997, updated 2003). EFSA has issued several opinions on the same product when used with dogs and cats (EFSA, 2004a and EFSA FEEDAP Panel 2013a), with chickens for fattening (EFSA FEEDAP Panel, 2010a) and with calves, lambs and kids for rearing and for fattening (EFSA FEEDAP Panel, 2013b). EFSA has also published a statement on the safety of this product when used in animal nutrition (EFSA FEEDAP Panel, 2010b). The Scientific Committee on Animal Nutrition (SCAN) issued two opinions on the safety of another product based on the same active agent (*E. faecium* NCIMB 10415/DSM 10663) for turkeys (EC, 2002), and for pigs for fattening, calves and chickens for fattening (EC, 1997, updated 2003). EFSA issued an opinion on the same product for dogs (EFSA, 2004b).

The Scientific Committee on Animal Nutrition (SCAN) issued one opinion on the use of a product based on *E. faecium* DSM 22502 s a feed additive (EC, 1997, updated 2003). EFSA issued also two opinions on the safety and efficacy of this product for chickens for fattening (EFSA, 2005) and for weaned piglets and calves (EFSA FEEDAP Panel, 2012a).

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 Enterococcus faecium (NCIMB 10415, DSM 22502, ATCC 53519 and ATCC 55593), when used under the conditions described in Table 1.

[17] Commission Regulation (EC) No 1333/2004 of 20 July 2004 concerning the permanent authorisation of certain additive in feedingstuffs. OJ L 247, 21.7.2004, p. 11.

[18] Commission (EC) No 600/2005 of 18 April 2005 concerning a new authorisation for 10 years of a coccidiostat as an additive in feedingstuffs, the provisional authorisation of an additive and the permanent authorisation of certain additives in feedingstuffs. OJ L 99, 19.4.2005, p. 5.

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

Additive	*Enterococcus faecium* (see Appendix for full names)
Registration number/EC No/No	-
Category of additive	Technological
Functional group(s) of additive	Silage additive

Description			
Composition, description	Chemical formula	Purity criteria	Method of analysis
See Appendix	Not appropriate	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 15789:2009 Identification method (genetic): PFGE

Trade name	Not appropriate
Name of the holder of authorisation	Not appropriate

Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period
		CFU/kg of complete feedingstuffs		
All animal species and categories	n.a.	See Appendix	n.a.	n.a.

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use	See Appendix
Specific conditions or restrictions for handling	For safety: eye protection and gloves shall be used during handling
Post-market monitoring	Not appropriate
Specific conditions for use in complementary feedingstuffs	Not appropriate

Maximum Residue Limit (MRL)			
Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues
Not appropriate	Not appropriate	Not appropriate	Not appropriate

ASSESSMENT

1. Introduction

Six genera of lactic acid-producing bacteria are commonly associated with forage species and collectively contribute to the natural ensiling process. This joint application made by a consortium of companies concerns four strains of *Enterococcus faecium*.

All strains are intended to be added individually to forages to promote ensiling (category: technological additive; functional group: silage additive) for eventual use of the silage in all animal species. All of these strains of *E. faecium* are currently authorised as zootechnical additives in the European Union.

2. Characterisation

The four strains that are the subject of this assessment 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.

Table 2: The strains of *Enterococcus faecium* and their accession numbers

Reference letter	Accession number(s)
A[19]	*Enterococcus faecium*—**NCIMB 10415**
B[20]	*Enterococcus faecium*—NCIMB 11118 —**DSM 22502**
C[21]	*Enterococcus faecium*—DSM 4788—**ATCC 53519**
D[22]	*Enterococcus faecium*—DSM 4789 —**ATCC 55593**

Accession numbers for which a copy of the certificate of deposition is provided are shown in bold.
DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen; ATCC, American Type Culture Collection; NCIMB, National Collection of Industrial and Marine Bacteria.

2.1. Identity and properties of the active agents

Strain A was isolated from the faeces of a healthy infant. It has not been genetically modified. The identification of the strain was based on DNA–DNA hybridisation against the type strain and on various phenotypic tests.[22] Strain-level identification was performed with various DNA-based techniques (e.g. strain-specific nested polymerase chain reaction (PCR) reaction, pulsed-field gel electrophoresis (PFGE) with *Apa*I and *Sma*I plasmid profiling) and phenotypic characterisation with, for example, phage typing and sugar fermentation patterns.[23]

Strain B was isolated from the gastrointestinal tract content of a healthy infant. It has not been genetically modified. Strain identification was based on a nearly complete 16S rRNA gene sequence, partial *atpA, rpoA* and *pheS* gene sequences and phenotypic characterisation with the analytical profile index (API). Genetic stability was shown by PFGE on several generations of the strain.[24]

Strains C and D were isolated from unspecified harvested plant material and neither has been genetically modified. Both strains were initially identified as *E. durans* based on a partial 16S rRNA gene sequence. However, the analysis of a partial *hsp60* gene sequence identified the two strains as

[19] Technical dossier/Section II/Annex 2-2-9 Agri-King.
[20] Technical dossier/Section II/Annex 2-2-11 Chr Hansen.
[21] Technical dossier/Section II/Annex 2-2-12 Pioneer.
[22] Technical dossier/Section II/Annex 2-2-23.
[23] Technical dossier/Section II and Supplementary information January 2013/Annexes 2-2-5 Agri-King and Qi_Agriking_E faecium NCIMB 10415_identification.
[24] Technical dossier/Section II/Annex 2-2-7 Chr Hansen.

E. faecium. Phenotypic characterisation was performed with sugar fermentation patterns. Strain-level differentiation was performed with PFGE with *Sma*I and *Csp*I; no genetic stability was shown.[25]

2.1.1. Antibiotic susceptibility

The susceptibility of the strains to the antibiotics recommended by the FEEDAP Panel in its Technical Guidance on the update of the criteria used in the assessment of bacterial resistance to antibiotics of human or veterinary importance (EFSA FEEDAP Panel, 2012b) was tested by a broth dilution method in Iso-Sensitest (IST) or Mueller–Hinton medium.

The minimum inhibitory concentration (MIC) values for strain A are below or equal to the EFSA cut-off values, except for kanamycin and erythromycin, the MIC values of which are higher than the cut-off by a single dilution. This is within the normal variation around the mean and, thus, does not raise concerns for safety.[26]

The MIC values for strains B and C are below or equal to the cut-off values established by EFSA. Therefore, no further investigation is required.[27]

The MIC values for strain D are below or equal to the EFSA cut-off values except for erythromycin and clindamycin, the MIC values of which are higher than the cut-off by a single dilution. This is within the normal variation around the mean and, thus, does not raise concerns for safety.[28]

2.1.2. Virulence

All strains are susceptible to ampicillin (MIC $\leq$ 2 mg/L) and, as an analysis of the sequenced genomes did not detect the presence of the genetic determinants IS*16*, hyl_{Efm} and *esp*, it is considered that the strains do not belong to the so-called hospital clade typical of clinical isolates.[29]

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, in most cases, material safety data sheets (MSDS) are provided. Cells are then separated from the growth medium by centrifugation or micro-filtration, cryoprotectants are added and the cell mix is finally freeze dried and ground. The ground powder is blended with sufficient carrier to meet the minimum specified concentration for each additive. Since the subject of the authorisation is the microbial agent, different formulations can be placed on the market with reference to that authorisation. Table 3 reports the minimum specified content and analysed content of the active agent for each additive. It also illustrates some formulations proposed by the applicant, some of which are defined as water soluble; others as in granular/dry form.

[25] Technical dossier/Section II/Annex 2-2-8 Pioneer.
[26] Technical dossier/Section II/Annex 2-2-23 Agri-King.
[27] Technical dossier/ Section II and Supplementary information January 2013/Annexes 2-2-21, 2-2-26 Pioneer and Qiia_Chr Hansen_E faecium DSM 22502_additional MICs.
[28] Technical dossier/Section II and Supplementary information January 2013/Annexes 2-2-26 Pioneer and Qiic_Pioneer_E faecium ATCC 55593_antibiotic resistance determinants.pdf.
[29] Technical dossier/ Section II and Supplementary information January 2013/ Annexes 2-2-19 and Qiii_Pioneer_E faecium ATCC 55593 and ATCC53519_Virulence factors_hospital associated strains_esp.

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

Additive	Formulation [(a)]	Minimum guaranteed cell count (CFU/g)	Mean cell count (CFU/g)
A[30]	Saccharose (88 %) Cellulose derivate (10 %)	1×10^{10}	5.4×10^{10} (*n* = 5, CV % 11)
B[31]	Maltodextrin (40–60 %) Silica (8 %)	1×10^{11}	5.7×10^{11} (*n* = 5, CV % 14)
C[32]	Maltodextrin	1×10^{10}	3.4×10^{11} (*n* = 4, CV % 15)
D[33]	Maltodextrin	1×10^{10}	3.2×10^{11} (*n* = 5, CV % 12.5)

(a): Granular/dry form of additives C and D: limestone (92–95 %) and silica (1–2 %).
CV, coefficient of variation.

The additives are routinely monitored for microbial contamination. Limits are set for yeasts and filamentous fungi (< 10^3 CFU/g additive), coliforms (< 10^3 CFU/g additive), *Escherichia coli* (< 10 CFU/g additive) and *Salmonella* (absence in 25 g of additive). Data from three batches of each additive confirmed compliance with these limits.[33] Given the nature of the fermentation medium and the food-grade excipients, the probability of contamination with heavy metals or mycotoxins is low and apparently not included in routine monitoring. Three batches from additive A and one batch from additives B, C and D were, however, sent for the analysis of aflatoxins B_1, B_2, G_1 and G_2.[34] The values obtained were either below the detection limit of the analytical method (< 1.0 µg/kg) or substantially lower (0.05 µg/kg) than the action limit set (1.0 µg/kg additive).

The available measurements of particle size distribution (two or three batches per product), obtained by laser diffraction, and dusting potential, obtained using a Stauber–Heubach dustometer, are summarised in Table 4. It should be noted that the applicant has provided the same data for strains C and D with no indication of the strain identity in the product batches tested.

When a microbial agent is authorised for use as a silage additive, different formulations can be placed on the market with reference to that authorisation; thus, the FEEDAP Panel notes that different formulations of the products might have different particle size distributions and dusting potential.

Table 4: Particle size and dusting potential

Strain	Particle size (water soluble form) [(a)] (%)	Dusting potential (g/m^3)
A[35]	5.7 < 10 µm; 29.2 < 50 µm	Dry/granular: 0. Water soluble: 18.3
B[36]	10 < 10 µm; 27.9 < 50 µm	0.5–13.6 [(b)]
C and D[37]	27.3 < 50 µm	Dry/granular: 4.2. Water soluble: 37.9

(a): In the granular formulations of all four strains, < 0.4 % of particles are < 50 µm in size.
(b): Three different formulations.

[30] Technical dossier/Section II/Annexes 2-1-10 and 158 Agri-King.
[31] Technical dossier/Section II/Annexes 2-1-12 and 17 Chr Hansen.
[32] Technical dossier/Section II/Annexes 2-1-13, 14 and 18 Pioneer.
[33] Technical dossier/Section II/Annexes 2_1_19-22.
[34] Technical dossier/Section II/Annexes 2_1_23-26.
[35] Technical dossier/Section II/Annexes 2_1_27; 2_1_34.
[36] Technical dossier/Section II/Annexes 2_1_29; 2_1_36.
[37] Technical dossier/Section II and Supplementary information January 2013/Annexes 2.1.30, 2.1.32 and 2.1 .37.

2.3. Stability

Based on studies supplied, the shelf-life of strains B, C and D in the sealed, moisture-tight containers in which they are supplied is 12 months and that of strain A is five months when stored at ambient temperature of 20–27 °C. In addition, under refrigeration (4–5 °C), shelf-life is 13 months for strains C and D, 18 months for strain B and 23 months for strain A.[38]

All strains were stable in water at ambient temperatures (20–27 °C) for a minimum period of 48 hours.[39]

2.4. Conditions of use

The additives are intended for use with all forages at the recommended doses shown in Table 5. Granulated products are intended to be directly applied to material for ensiling whereas all other formulations are intended to be first suspended in water and then distributed by spraying.

Table 5: Recommended doses

Strain	Recommended dose (CFU/kg fresh silage)
A, NCIMB 10415	1×10^8
B, DSM 22502	1×10^8
C, ATCC 53519	1×10^7
D, ATCC 55593	5×10^6

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 Appendix B.

3. Safety

3.1. Safety for the target animals

E. faecium occurs as a normal constituent of silage and is often present in forages in numbers equivalent to those delivered by silage additives. It is not expected that the use of the four *E. faecium* strains at the doses proposed would substantially increase the exposure to the animals given silage as part of their rations. Therefore, the FEEDAP Panel considers that use of these strains in the preparation of silage is safe for the target animals.

3.2. Safety for the consumers

None of the *E. faecium* strains contains marker genes typical of hospital-associated isolates responsible for clinical infections, and all are susceptible to clinically relevant antibiotics. The metabolic pathways of *E. faecium* are well known and, when the potential for infection is excluded, no other harmful metabolites or substances are expected to be produced during fermentation. In addition, cells are isolated from the spent medium prior to incorporation in the additive and, therefore, any carry-over from the fermentation would be negligible.

None of the additives contains excipients of concern (section 2.2). Consequently, the FEEDAP Panel does not see the need for toxicological studies.

[38] Technical dossier/Section II and Supplementary information January 2013/Annexes 2_4_1-4 and New Data_Chr Hansen_E faecium DSM 22501_shelf-life report.
[39] Technical dossier/Supplementary information January 2013/Annexes Qv.

3.3. Safety for the user

No data are available on skin/eye irritation or skin sensitisation. Therefore, the additives should be considered to have the potential to be skin and eye irritants and skin sensitisers and should be treated accordingly.

The dusting potential of some of the preparations 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. Although users at the farm level are exposed to the additive for only a short period of time when preparing the aqueous suspension or when applying the additive to forage, given the proteinaceous nature of the active agents, the additives should be considered to have the potential to be respiratory sensitisers and treated accordingly.

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 cryoprotectants and carriers which 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 focus, provided that other components do not introduce concerns. The excipients listed (section 2.2) would not introduce additional risks.

3.4. Safety for the environment

Enterococci are naturally present in silage and are part of the indigenous microbiota of the digestive tract of humans and animals and are thus ubiquitous in the environment. As the use of these strains in the preparation of silage would not measurably increase the environmental burden, no further assessment of environmental safety is considered necessary.

4. Efficacy

In some studies, statistical significance was seen only when the second decimal place was considered. Generally, changes of this magnitude are considered of little or no biological relevance and so were not considered.

4.1. *Enterococcus faecium* NCIMB 10415 (strain A)

Three studies were carried out, the first with 133-L barrels and the second with mini-silos (0.7 L). In the third study, mini-silos were used but the volume was not specified. Forages used fulfilled the definition of easy, moderately difficult and difficult to ensile material as specified in Regulation (EC) No 429/2008 (Table 6). The duration of the experiments was 90 days. In each case, the contents of three replicate silos (four in study 2) were sprayed with the additive at a dose of 1×10^8 CFU/kg forage (confirmed by analysis). Forage for the control silos was sprayed with an equal volume of water but without the additive. Ambient temperature was controlled at 22 °C.

Table 6: Characteristics of the forage samples used in the ensiling experiments

Study no	Test material	Dry matter content (% fresh material)	Water-soluble carbohydrate content (% fresh material)
1[40]	Lucerne	56.0	6.3
2[41]	Ryegrass	12.2	1.8
3[42]	Grass	18.0	1.4

[40] Technical dossier/Supplementary information March 2013/Annex_Qi_Agriking_ E. faecium NCIMB 10415_Raw Data_Easy to Ensile.
[41] Technical dossier/ Supplementary information March 2013/ Annex_Qi_Agriking_ E. faecium NCIMB 10415_Raw Data_SILAC_ Moderately Difficult to Ensile.
[42] Technical dossier/ Supplementary information March 2013/Annex_Qi_Agriking_ E. faecium NCIMB 10415_Raw Data_ Difficult to Ensile.

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 concentrations, ethanol, ammonia and total nitrogen (Table 7). Data were analysed using a non-parametric Kruskal–Wallis test with chi-squared approximation.

Table 7: Summary of the analysis of ensiled material recovered at the end of the experiments (90 days) with *Enterococcus faecium* NCIMB 10415 (strain A)

Study no	Treatment (CFU/kg)	Dry matter loss (%)	pH	Lactic acid (% ensiled matter)	Acetic acid (% ensiled matter)	Ammonia-N (% total N)
1	0	1.0	5.5	0.7	0.2	7.4
	1×10^8	0.6*	5.3*	1.4*	0.1	7.0
2	0	7.9	4.1	1.5	0.6	12.5
	1×10^8	3.4	4.1	2.0*	0.3*	9.7*
3	0	1.0	4.2	1.0	1.2	17.9
	1×10^8	0.5*	4.2	1.5*	0.3*	10.4*

*Significantly different to the control at $P < 0.05$.

The addition of strain A consistently increased the concentration of lactic acid and decreased the concentration of acetic acid. Although pH was only significantly affected in one study, the low value in control samples did not provide a margin for improvement. Dry matter preservation was significantly improved in two out of three studies and numerically improved in the third. In addition, benefits were seen in terms of reduced protein breakdown in two out of three studies. Taken overall, the data indicate that strain A has the potential to improve the production of silage from easy, moderately difficult and difficult to ensile materials.

4.2. *Enterococcus faecium* DSM 22502 (strain B)

Eight studies were performed with 3-L mini-silos and forages fulfilling the definition of easy, moderately difficult and difficult to ensile materials as specified in Regulation (EC) No 429/2008 (Table 8). The contents of five replicate silos were sprayed with the additive at a dose of 1×10^8 CFU/kg forage (confirmed by analysis). Forage for the control silos was sprayed with an equal volume of water but without the additive. Ambient temperature was controlled at 20 °C.

Replicate silos were opened at 90 days (95, 97 and 99 days in studies 6, 7 and 8 respectively) after ensiling and the contents were analysed for dry matter content, pH, lactic and volatile fatty acids concentrations, alcohols, ammonia and total nitrogen (Table 9).

Data were analysed by non-parametric Wilcoxon and Kruskal–Wallis tests followed by chi-squared approximation.

Table 8: Characteristics of the forage samples used in the ensiling experiments

Study no	Test material	Dry matter content (% fresh material)	Water-soluble carbohydrate content (% fresh material)
1[43]	Whole crop maize	32.8	3.6
2[44]	Red clover/ryegrass (70:30)	31.7	2.9
3[45]	Lucerne cv. Europa	31.4	1.2
4[34]	Lucerne cv. Birute	32.2	1.2
5[34]	Lucerne cv. Verko	35.0	1.2
6[46]	Lucerne cv. FSG 408	17.2	0.2
7[35]	*Fodder galega* cv. Gale	14.3	0.1
8[35]	Lucerne cv. Jõgeva 118	13.5	0.1

Table 9: Summary of the analysis of ensiled material recovered at the end of the experiments (90–99 days) with *Enterococcus faecium* DSM 22502 (strain B)

Study no	Treatment (CFU/kg)	Dry matter loss (%)	pH	Lactic acid (% ensiled matter)	Acetic acid (% ensiled matter)	Ammonia-N (% total N)
1	0	7.4	4.0	0.9	0.8	6.1
	1×10^8	4.0*	3.7*	1.4*	0.7*	3.6*
2	0	10.2	4.7	0.8	0.8	5.7
	1×10^8	5.0*	4.1*	1.5*	0.8	2.6*
3	0	6.7	5.5	0.3	0.8	11.1
	1×10^8	5.1*	5.4*	1.6*	1.0*	9.2*
4	0	6.9	5.2	0.6	1.1	10.4
	1×10^8	4.6*	4.9*	1.8*	1.1	9.0*
5	0	6.8	5.3	0.8	1.3	9.1
	1×10^8	4.3*	4.8*	2.0*	1.2	7.7*
6	0	9.9	6.0	0.2	0.6	18.5
	1×10^8	9.3	6.0	0.2	0.6	22.5*
7	0	9.5	6.0	0.1	0.4	23.7
	1×10^8	10.5	5.8*	0.2	0.7*	23.0
8	0	10.1	6.5	0.3	0.7	32.7
	1×10^8	9.9	6.4*	0.2	0.9*	31.1

*Significantly different to the control at $P < 0.05$.

The assessment of the data is based on the first five studies. The three remaining studies used forage materials with a very low water-soluble carbohydrate content and would not normally be considered suitable for ensiling.

The results indicated that strain B consistently and significantly increased lactic acid production with a consequent reduction in final pH and dry matter loss. Ammonia nitrogen was also significantly reduced in all five studies implying a better preservation of protein. This was shown using easy, moderately difficult and difficult to ensile materials.

[43] Technical dossier/Section IV and Supplementary information January 2013/Annexes 4_3 and Annex_new data_Chr Hansen_E faecium_DSM 225052_easy_Trial 80059 easy.
[44] Technical dossier/ Supplementary information January and March 2013/Annexes new data_Chr Hansen_E faecium_DSM 225052_moderate_Trial 80046 and Qii Chr Hansen E faecium NCIMB 11161 Efficacy trial moderately difficult.
[45] Technical dossier/Section IV and Supplementary information January 2013/Annexes 4_3 and Annex_new data_Chr Hansen_E faecium_DSM 225052_ difficult_Trial 80002.
[46] Technical dossier/Section IV and Supplementary information January 2013/Annexes 4_3 and Annex_new data_Chr Hansen_E faecium_DSM 225052_ difficult_Trial 80006.

4.3. *Enterococcus faecium* ATCC 53519 and ATCC 55593 (strains C and D)

Five studies were carried out with mini-silos of 2.8 L and forages fulfilling the definition of easy, moderately difficult and difficult to ensile materials as specified in Regulation (EC) No 429/2008 (Table 10). The contents of four replicate silos were sprayed with the additive at a dose of 1×10^7 CFU/kg forage (strain C, not confirmed by analysis) or 5×10^6 CFU/kg forage (strain D, not confirmed by analysis). Forage for the control silos was sprayed with an equal volume of water but without the additive. Conditions of storage were not reported.

Table 10: Characteristics of the forage samples used in the ensiling experiments with strains C and D

Study no	Test material	Dry matter content (% fresh material)	Water-soluble carbohydrate content (% fresh material)
1[47]	Ryegrass	41.5	7.8
2	Ryegrass	36.9	6.1
3	Ryegrass	39.0$^{\xi}$	8.4 (a)
4[48]	Lucerne	42.2	2.3
5[49]	Maize	72.9	0.6

(a): Slightly different values for dry matter and water-soluble carbohydrate contents are reported for the same material depending on the additive applied. The values showed in the table are for strain C only.

Replicate silos were opened at 90 days after ensiling and the contents were analysed for dry matter content, pH, lactic and volatile fatty acids concentrations, alcohol and ammonia-N content (Tables 11 and 12).

Data were analysed by trial based on a model that included a fixed effect of treatment and an error term. This was then followed by pair-wise comparison of least-squares means of treatments with their corresponding control based on an *F*-test and a *t*-test of significance. Normality of the data was analysed by histograms of residuals (*Q–Q* plot analysis).

Table 11: Summary of the analysis of ensiled material recovered at the end of the experiments (90 days) with *Enterococcus faecium* ATCC 53519 (strain C)

Trial	Treatment (CFU/kg)	Dry matter loss (%)	pH	Lactic acid (% ensiled matter)	Acetic acid (% ensiled matter)	Ammonia-N (% total N)
1	0	1.9	3.9	3.2	1.3	7.9
	1×10^7	1.9	3.9	3.5*	1.3	7.8
2	0	2.6	5.0	1.0	0.2	7.9
	1×10^7	2.8	4.6*	1.5*	0.5*	8.4
3	0	1.9	4.7	1.7	0.5	8.2
	1×10^7	1.8	4.5*	2.4*	0.5	8.1
4	0	n.d.	4.6	1.7	0.5	3.3
	1×10^7		4.5*	2.2*	0.7*	3.2
5	0	2.8	4.0	0.6	0.1	2.4
	1×10^7	2.6	3.9*	0.9*	0.1	2.7*

n.d, no data available.
*Significantly different to the control at $P < 0.05$.

[47] Technical dossier/Section IV and Supplementary information January 2013/Annexes 4_4 and Qviii_Pioneer_E. faecium ATCC 53519 and ATCC55593_Efficacy.
[48] Technical dossier/Supplementary information January 2013/Annex_Qviii_Pioneer_E. faecium ATCC 53519 and ATCC55593_Efficacy_moderate.pdf
[49] Technical dossier/Supplementary information January 2013/Annex_Qviii_Pioneer_E. faecium ATCC 53519 and ATCC55593_Efficacy_difficult.pdf

The effects seen with easy, moderately difficult and difficult to ensile materials are marginal. A small but significant increase in lactic acid production was seen in all studies with a concomitant reduction in pH in four out of five studies. However, there was no significant change in dry matter loss or evidence of reduced protein breakdown.

Table 12: Summary of the analysis of ensiled material recovered at the end of the experiments (90 days) with ATCC 55593 (strain D)

Trial	Treatment (CFU/kg)	Dry matter loss (%)	pH	Lactic acid (% ensiled matter)	Acetic acid (% ensiled matter)	Ammonia-N (% total N)
1	0	1.9	3.9	3.2	1.3	7.9
	5×10^6	1.8*	3.8*	3.7*	1.2*	7.8
2	0	2.6	5.0	1.0	0.2	7.9
	5×10^6	2.1*	4.6*	2.0*	0.2	8.1
3	0	2.8	4.4	2.6	0.3	8.4
	5×10^6	1.1*	4.4	2.8	0.3	8.3
4	0	n.d.	4.6	1.7	0.5	3.3
	5×10^6		4.5*	2.3*	0.7*	3.2
5	0	2.8	4.0	0.6	0.1	2.4
	5×10^6	3.1	3.9* [(a)]	0.9*	0.1	3.0*

n.d., no data available.
*Significantly different to the control at $P < 0.05$.

Results for strain D resemble those for strain C, although, in the case of strain D, there was evidence of significant preservation of dry matter with easy to ensile materials.

CONCLUSIONS

None of the four *Enterococcus faecium* strains (NCIMB 14015, DSM 22502, ATTC 53510 and ATTC 55593) was shown to contain marker genes typical of hospital-associated isolates responsible for clinical infections and all were susceptible to clinically relevant antibiotics. In addition, no other sources of concern have been identified in the additives. Consequently, the FEEDAP Panel considers the use of these *E. faecium* strains as silage additives safe for consumers of animal products.

It is not expected that the use of *E. faecium* at the doses proposed would substantially increase the exposure of animals given silage as part of their rations. Therefore, the FEEDAP Panel considers that the use of these strains in the preparation of silage is safe for the target animals.

In the absence of evidence, these additives should be regarded as skin and eye irritants and potential skin sensitisers. Given the proteinaceous nature of the active agents, the FEEDAP Panel considers it prudent to treat these additives as respiratory sensitisers. Given the high dusting potential of most of the preparations tested, there is a need to take measures to minimise inhalation exposure of workers.

The use of these strains as silage additives is considered safe for the environment.

E. faecium strains NCIMB 10415 (A) and DSM 22502 (B) have the potential to improve the production of silage from easy, moderately difficult and difficult to ensile forage materials at a minimum dose of 1×10^8 CFU/kg fresh material.

Given the magnitude of the responses recorded and the absence of any substantive evidence of nutrient preservation, the data for the two *E. faecium* strains ATCC 53519 (C) and ATCC 55393 (D), taken overall, show little evidence of a benefit when these strains are used in the production of silage.

DOCUMENTATION PROVIDED TO EFSA

1. *Enterococcus faecium* (NCIMB 10415, DSM 3530 and 22502, ATCC 53519 and 55593). September 2010. Submitted by SILAC EEIG.

2. *Enterococcus faecium* (NCIMB 10415, DSM 3530 and 22502, ATCC 53519 and 55593). Supplementary information. Month year. Submitted by SILAC EEIG. *Enterococcus faecium* (NCIMB 10415, DSM 3530 and 22502, ATCC 53519 and 55593). Supplementary information. January 2013. Submitted by SILAC EEIG.

3. *Enterococcus faecium* (NCIMB 10415 and 22502, ATCC 53519 and 55593). Supplementary information. March 2013. Submitted by SILAC EEIG.

4. *Enterococcus faecium* (NCIMB 10415 and 22502, ATCC 53519 and 55593). Supplementary information. May 2013. Submitted by SILAC EEIG.

5. Evaluation report of the European Union Reference Laboratory for Feed Additives on the methods of analysis for *Enterococcus faecium* (NCIMB 10415, DSM 3530 and 22502, ATCC 53519 and 55593).

6. Comments from Member States received through the ScienceNet.

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EFSA Panel on Additives and Products or Substances Used in Animal Feed (FEEDAP), 2010b. Statement on the assessment of the safety of Cylactin® (*Enterococcus faecium*) used in animal nutrition. EFSA Journal 2010;8(12):1955, 14 pp. doi:10.2903/j.efsa.2010.1955

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), 2012a. Scientific Opinion on Lactiferm® (*Enterococcus faecium*) as a feed additive for weaned piglets and calves. EFSA Journal 2012;10(2):2574, 15 pp. doi:10.2903/j.efsa.2012.2574

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EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), 2013a. Scientific Opinion on the safety and efficacy of Cylactin® (*Enterococcus faecium*) as a feed additive for cats and dogs. EFSA Journal 2013;11(2):3098, 15 pp. doi:10.2903/j.efsa.2013.3098

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), 2013b. Scientific Opinion on Cylactin® (*Enterococcus faecium*) for calves, lambs and kids for rearing and for fattening. EFSA Journal 2013;11(2):3097, 14 pp. doi:10.2903/j.efsa.2013.3097

APPENDICES

Appendix A. Appendix to Table 1: SILAC EEIG – *Enterococcus faecium* dossier

Table 13: List of feed additives included in the dossier

Name of authorisation holder	Name of the additive as detailed in the Community Register of Feed Additives pursuant to Regulation (EC) No 1831/2003	Name as proposed by the applicant
Agri-king Ltd.	*Enterococcus faecium* NCIMB 10415	*Enterococcus faecium* NCIMB 10415
Chr. Hansen A/S	*Enterococcus faecium* M74 – CCM 6226 – NCIMB 11181	*Enterococcus faecium* CCM 6226 – NCIMB 11181 – DSM 22502
Pioneer Hi-Bred Int., Inc.	*Enterococcus faecium* SF202 – DSM 4788 – ATCC 53519	*Enterococcus faecium* ATCC 53519
Pioneer Hi-Bred Int., Inc.	*Enterococcus faecium* SF301 – DSM 4789 – ATCC 55593	*Enterococcus faecium* ATCC 55593

Table 14: Detailed description of the additives

Category and functional group	Subclassification	Additive	Composition, chemical formula, description, analytical method[50]	Species or category of animal	Maximum age	Minimum content	Maximum content	Other provisions	Maximum residue limits in the relevant foodstuffs of animal origin	End of period of authorisation
						CFU/kg of complete feedingstuffs, supplementary feed (based on end feed)				
1. Technological additives, k. Silage additives	2. Microorganisms	*Enterococcus faecium* NCIMB 10415 (name of authorisation holder: Agri-king Ltd)	Preparation of *Enterococcus faecium* NCIMB 10415 having minimum activity of: 1×10^{10} CFU/g. Enumeration method: EN15788:2009. Identification method: PFGE	All animals species and categories	–	1×10^{8} (easy, moderate forage)	–	1. Recommended dosage in silage: 1×10^{8} CFU/kg fresh forage for silage preparation 2. In the direction for use indicate the storage temperature, and storage life. 3. For safety: eye protection and gloves shall be used during handling	–	To be assigned

[50] Available on the EURL website.

1. Technological additives, k. Silage additives	2. Microorganisms	*Enterococcus faecium* DSM 22502 (name of authorisation holder: Chr. Hansen A/S)	Preparation of *Enterococcus faecium* DSM 22502 having minimum activity of: 1×10^{11} CFU/g. Enumeration method: EN15788:2009. Identification method: PFGE	All animals species and categories	–	1 x1 0^{8} (easy, moderate, difficult forage)	–	1. Recommended dosage in silage: 1×10^{8} CFU/kg fresh forage for silage preparation 2. In the direction for use indicate the storage temperature, and storage life. 3. For safety: eye protection and gloves shall be used during handling	–	To be assigned
1. Technological additives, k. Silage additives	2. Microorganisms	*Enterococcus faecium* ATCC 53519(name of authorisation holder: Pioneer Hi-Bred Int., Inc.)	Preparation of *Enterococcus faecium* ATCC 53519 having minimum activity of: 1×10^{10} CFU/g. Enumeration method: EN15788:2009. Identification method: PFGE	All animals species and categories	–	1×10^{7} (easy forage)	–	1. Recommended dosage in silage: 1×10^{7} CFU/kg fresh forage for silage preparation 2. In the direction for use indicate the storage temperature, and storage life. 3. For safety: eye protection and gloves shall be used during handling	–	To be assigned

1. Technological additives, k. Silage additives	2. Microorganisms	*Enterococcus faecium* ATCC 55593 (name of authorisation holder: Pioneer Hi-Bred Int., Inc.)	Preparation of *Enterococcus faecium* ATCC 55593 having minimum activity of: 1×10^{10} CFU/g. Enumeration method: EN15788:2009. Identification method: PFGE	All animals species and categories	–	5×10^{6} (easy forage)	–	1. Recommended dosage in silage: 5×10^{6} CFU/kg fresh forage for silage preparation 2. In the direction for use indicate the storage temperature, and storage life. 3. For safety: eye protection and gloves shall be used during handling	–	To be assigned

PFGE: pulsed field gel electrophoresis.

Appendix B. Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Methods of *Enterococcus faecium* (NCIMB 10415, DSM 3530[51] and 22502, ATCC 53519 and 5559)[52]

This report is on the evaluation of feed additives "*micro-organisms used as silage agents"*, which is related to the application of fifteen *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.[53] The intended use of the current applications is for all animal species, except for FAD-2010-0387, for which bovines, ovines, pigs, poultry, rabbits, horses and goats are specified.

For identification and characterisation of all fifteen *micro-organisms* of concern (i.e. *enterococci, 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 Bile Esculin Azide agar (EN 15788) for *enterococci*;
- 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 fifteen *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.

[51] The withdrawal of the request for the authorisation of this strain was submitted after the Report had been issued.
[52] The EURL produced a combined report for *Enterococcus faecium, Lactobacillus buchneri, L. plantarum,* and *Pediococcus pentosaceus*.
[53] Full list provided in EURL evaluation report, available from the EURL website: http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-uorg3.pdf