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SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of *Lactobacillus plantarum* (NCIMB 40027) as a silage additive for all animal 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

The strain of *Lactobacillus plantarum* is intended to improve the ensiling process at proposed doses ranging from 1×10^8 to 1×10^9 CFU/kg fresh material. This species is considered by EFSA to be suitable for the qualified presumption of safety approach to safety assessment. As the identity of the strain has been established and as no antibiotic resistance of concern was detected, the use of the strain in the production of silage is presumed safe for livestock species, for consumers of products from animals fed the treated silage and for the environment. The active agent is not an eye or skin irritant or a skin sensitiser. Given the proteinaceous nature of the active agent and the high dusting potential of the product tested, the FEEDAP Panel considers it prudent to treat this additive as a respiratory sensitiser. A total of 20 laboratory-scale ensiling studies were conducted with *L. plantarum* applied at 1×10^9 or 1×10^8 CFU/kg forage. In all the studies, forage containing the additive was compared with untreated control materials, and the duration of the studies was at least 90 days. *L. plantarum* has the potential to improve the production of silage by increasing lactic acid content and the preservation of dry matter, by reducing the pH and protein degradation. This was demonstrated in a range of easy and moderately difficult to ensile forage materials at a minimum concentration of 1×10^8 CFU/kg fresh material and also in difficult to ensile forage materials when added at a concentration of 1×10^9 CFU/kg fresh material.

KEY WORDS

Technological additive, silage additive, *Lactobacillus plantarum*, QPS, safety, efficacy

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[2] Panel members: Gabriele Aquilina, Alex Bach, Vasileios Bampidis, Maria De Lourdes Bastos, Lucio Guido Costa, Gerhard Flachowsky, Mikolaj Antoni Gralak, Christer Hogstrand, Lubomir Leng, Secundino López-Puente, Giovanna Martelli, Baltasar Mayo, Fernando Ramos, Derek Renshaw, Guido Rychen, Maria Saarela, Kristen Sejrsen, Patrick Van Beelen, Robert John Wallace and Johannes Westendorf. Correspondence: FEEDAP@efsa.europa.eu

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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 a product based on a specific strain of *Lactobacillus plantarum*, when used as a technological additive intended to improve the ensiling process in the range of 1×10^8 to 1×10^9 CFU/kg fresh material.

The species *L. plantarum* is considered by EFSA to be suitable for the qualified presumption of safety approach to safety assessment. Therefore, it does not require any specific demonstration of safety other than confirming its susceptibility to antibiotics of human and veterinary clinical significance. As the identity of the strain has been clearly established and as no antibiotic resistance of concern was detected, the use of the strain in the production of silage is presumed safe for livestock species, for consumers of products from animals fed the treated silage and for the environment.

The active agent is not an eye or skin irritant or skin sensitiser. Although users at the farm level are exposed to the additive for only a short period of time when preparing the aqueous suspension, given the proteinaceous nature of the active agent and the high dusting potential of the product tested, the FEEDAP Panel considers it prudent to treat this additive as a respiratory sensitiser.

A total of 20 laboratory-scale ensiling studies were conducted with *L. plantarum* applied at 1×10^9 or 1×10^8 CFU/kg silage. In all studies, forage containing the additive was compared with untreated control materials, and the duration of the studies was at least 90 days. *L. plantarum* has the potential to improve the production of silage by increasing lactic acid content and the preservation of dry matter and by reducing the pH and protein degradation. This was demonstrated in a range of easy and moderately difficult to ensile forage materials at a minimum concentration of 1×10^8 CFU/kg fresh material and also in difficult to ensile forage materials when added at a concentration of 1×10^9 CFU/kg fresh material.

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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 Ecosyl Products Ltd.[5] for re-evaluation of the product *Lactobacillus plantarum* (NCIMB 40027) to be used as a feed additive 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.

This product was included in the European 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 product *Lactobacillus plantarum* (NCIMB 40027), 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] Ecosyl Products Ltd. Roseberry Court, Ellerbeck Way, Stokesley, TS9 5QT, United Kingdom.
[6] EFSA Dossier reference: FAD-2010-0259.

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

Additive	*Lactobacillus plantarum* NCIMB 40027
Registration number/EC No/No	-
Category(-ies) of additive	Technological additives
Functional group(s) of additive	Silage additive

Description			
Composition, description	Chemical formula	Purity criteria	Method of analysis
Lactobacillus plantarum NCIMB 40027 min. 1 x 10^{11} CFU/g	n.a.	Total contaminants < 10^6 CFU/g *Salmonella* absent in 25 g Yeasts < 10^2 CFU/g Moulds < 10^2 CFU/g Heavy metals as Pb < 10 mg/kg Pb < 5 mg/kg As < 3mg/kg Aflatoxin B1 < 0.05 mg/kg	BS ISO 15214:1998 – Microbiology of food and animal feedingstuffs – Horizontal method for the enumeration of mesophilic lactic acid bacteria – Colony count technique at 30°C

Trade name	n.a.
Name of the holder of authorisation	n.a.

Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period
		CFU/kg of complete feedingstuffs		
All species	Not applicable	1 x 10^8	1 x 10^9	

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use	-
Specific conditions or restrictions for handling	-
Post-market monitoring	-
Specific conditions for use in complementary feedingstuffs	-

Maximum Residue Limit (MRL)			
Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues
-	-	-	-

ASSESSMENT

1. Introduction

Six genera of lactic acid-producing bacteria, including *Lactobacillus* spp., are commonly associated with forage species and collectively contribute to the natural ensiling process. The present application concerns a strain of *Lactobacillus plantarum* to be added to forages to promote ensiling (technological additive, functional group: silage additive) for eventual use of the silage for all animal species. *L. plantarum* is considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to safety assessment (EFSA, 2007, 2012a). This approach requires the identity of the strain to be conclusively established and evidence that it does not show resistance to antibiotics of human and veterinary importance.

2. Characterisation

2.1. Identity and properties of the active agent

The *Lactobacillus plantarum* strain was isolated from silage and has been deposited in the National Collection of Industrial, Marine and Food Bacteria (NCIMB) with the accession number NCIMB 40027.[7] It has not been genetically modified. Taxonomic identification of strain NCIMB 40027 as *L. plantarum* was achieved by phenotypic tests and sequence analysis of the complete 16S rRNA gene.[8] Pulsed-field gel electrophoresis with *Asc*I and *Sfi*I is used as a strain-specific method of detection.[9] The same technique was used to assess genetic stability.

The strain was tested for antibiotic susceptibility using the broth microdilution method. The battery of antibiotics tested included those recommended by EFSA (EFSA, 2012b).[10] As all minimum inhibitory concentration values for the *L. plantarum* strain were below or equal to the cut-off values defined by the FEEDAP Panel, no further investigation is required.

2.2. Production and characteristics of the additive

The active agent is grown in a sterilised medium typical of those used for lactic acid bacteria and then separated from the growth medium by centrifugation. The resulting paste (18–22 % solids, w/w) is combined with a fixed weight of cryoprotectants (amounting to approximately 18–22 % glycine, 18–22 % sodium erythorbate and 20–24 % (w/w) of one of the following compounds: maltodextrin, sucrose, sweet whey, skimmed milk powder or anhydrous dextrose) allowing different formulations. The resulting mixture is then freeze dried, grounded and blended with sufficient sodium aluminosilicate to meet the minimum specification of 1×10^{11} CFU/g. Material safety datasheets are provided for cryoprotectants and carrier materials, all of which are of food grade and do not introduce safety concerns.

Data on 2 577 production batches showed that the minimum specification (1×10^{11} CFU/g) was exceeded in all cases (mean 3×10^{11} CFU/g additive).

The additive is routinely monitored for microbial contamination in the final product. Specifications are set for yeasts and filamentous fungi (<100 CFU/g additive) and *Salmonella* (absence in 25 g additive). Data from nine batches confirmed compliance with the set microbiological values.[11]

Given the nature of the fermentation medium and the food-grade excipients, the probability of contamination with heavy metals or mycotoxins is considered to be low and consequently not included

[7] Technical dossier/Section II/Annex II.12.2.2.1.d.
[8] Technical dossier/Section II/Annex II.13.2.2.1.e.
[9] Technical dossier/Supplementary information January 2013/Annexes 1–3.
[10] Technical dossier/Section II/Annex II.17.2.2.2.c.
[11] Technical dossier/Section II/Annex I.20.2.4.1.c.

in routine monitoring. Three batches of the additive were, however, sent for analysis to confirm this position. Values for aflatoxins B_1, B_2, G_1 and G_2 and deoxynivalenol were <0.2, <0.1, <0.2 and <0.1 µg/kg, respectively. Those for lead, mercury, cadmium and arsenic were <1, <2, <1 and <4 mg/kg, respectively. Zearalenone concentrations were in the range 53–227 µg/kg.[12]

Three batches of the additive were examined for particle size distribution by laser diffraction.[13] The average median particle size was 255.7 µm, with 14 % by volume of the additive consisting of particles with a diameter below 50 µm and 6 % of particles having a diameter below 10 µm. The same three samples of the product were used to measure the dusting potential with a Heubach dustometer.[14] The mean value for dust emission was 0.8 %, which approximates to 2 g/m^3. Other formulations of the product might have different particle size distribution and dusting potential.

2.3. Stability

The shelf-life of three batches of the additive in the sealed packaging in which they are supplied was studied and shown to be at least 12 months when stored at -10 °C and at 25°C.[15] Moreover, stability over 12 months of three different formulations has been demonstrated at 25 °C.

Short-term stability in water was determined in a study including three batches of a premixture of additives including the one under assessment suspended in water in concentrations mimicking the proposed application rate and stored under ambient conditions (23 °C).[16] Bacterial counts were made at time intervals up to 96 hours. The additive showed little or no loss after 72 hours.

2.4. Conditions of use

The additive is intended for use with forages at a minimum dose of 1.0×10^8 and a maximum dose of 1.0×10^9 CFU/kg fresh matter.

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 agent 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 susceptibility qualification has been met and the identity of the strain established. Consequently, *L. plantarum* NCIMB 40027 is suitable for the QPS approach to safety assessment and no further assessment of safety for the target species, consumers of products from animals fed treated silage or the environment would be required. However, this conclusion can be extended to the additive only provided that no other sources of concern are identified.

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 sodium erythorbate and glycine as cryoprotectants for use in the production of the additive. The Panel notes that these compounds are authorised for use in the EU as food additives but not as feed additives.

A study of acute dermal irritation/corrosion with a freeze-dried culture of *L. plantarum* NCIMB 40027 was performed following OECD Guideline 404.[17] Over an observation period of 72 hours, the test

[12] Technical dossier/Section II/Annex II.1.2.1.4.a.
[13] Technical dossier/Section II/Annex II.4.2.1.5.a.
[14] Technical dossier/Section II/Annex II.6.2.1.5.c.
[15] Technical dossier/Section II/Annex II.18.2.4.1.a.
[16] Technical dossier/Section II/Annex II.22.2.4.1.e.
[17] Technical dossier/Section III /Annex III.12.3.3.1.c.

material produced mild irritation to rabbit skin which did not meet the criteria for classification as irritant or corrosive according to Commission Directive 2001/59/EC.[18]

A study of the acute eye irritancy of a freeze-dried culture of *L. plantarum* NCIMB 40027 was carried out in three male New Zealand White rabbits and following OECD Guideline 405.[19] The results showed that the active agent caused transient inflammatory responses in the eyes of all treated rabbits, but the responses did not meet the criteria for classifying the additive as irritant or corrosive according to Commission Directive 2001/59/EC.

The results of a local lymph node assay in mice carried out according to OECD Guideline 429 indicated that *L. plantarum* NCIMB 40027 is not a potential skin sensitiser.[20]

The dustiness of the preparation tested indicated a potential for users to be exposed via inhalation. Given the proteinaceous nature of the active agent, the additive should be considered to have the potential to be a respiratory sensitiser 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 several cryoprotectants and carriers which 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 focus provided that other components do not introduce concerns. Most of the excipients listed (Section 2.2) would not be expected to introduce additional risks to their conventional use.

4. Efficacy

A total of 20 ensiling studies are described. In 11 (studies 1–11),[21] *L. plantarum* NCIMB 40027 was applied at a dose of 1×10^9 CFU/kg silage. In the other nine (studies 12–20)[22] it was applied at a dose of 1×10^8 CFU/kg/silage. In all studies, forage to which the additive had been applied was compared with untreated control materials, and the duration of the studies was 90 days except for studies 1 (120 days), 2 (244 days), 13 (210 days), 15 (94 days), 16 (94 days) and 20 (not specified).

Forages of different botanical origin and different dry matter (DM) and water-soluble carbohydrate contents were ensiled, representing materials easy (studies 1, 2, 3, 4, 12, 13, 14, 15, 16, 17 and 18), moderately difficult (studies 5, 6, 7, 8, 9, 10, 19 and 20) and difficult (study 11) to ensile as defined in Regulation (EC) No 429/2008 (see Table 2).

Mini- and micro-silos of different capacity were used: buckets of 1 L in study 20, buckets of 5 L in studies 1, 2, 11, 12, 13, 14 and 16, buckets of 19 L in studies 4, 5 and 6, Weck jars of 1.5 L in studies 3, 7, 8, 9, 10 and 15, and laboratory silos of 1 L in studies 17 and 18. In study 19, laboratory silos of unspecified size were used. In most cases, three replicates were used for control and treated silages, except for studies 11 (four replicates), 12 (five replicates), 17 (six replicates) and 20 (15 replicates). In each case, the contents of the silos were sprayed with the additive dissolved in 10 mL water/kg fresh forage. Forage for the control silos was sprayed with an equal volume of water but without the additive. The silos were stored at ambient temperature ranging between 20 and 25 °C.

Replicate silos were opened at the end of the experiment and the contents were analysed for DM content, pH, lactic and volatile fatty acids (VFAs) concentrations, ethanol, ammonia and total nitrogen. DM losses during fermentation were determined in 14 of the studies. Statistical analysis was

[18] Commission Directive 2001/59/EC of 6 August 2001 adapting to technical progress for the 28th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. OJ L 255, 21.8.2001, p. 1.
[19] Technical dossier/Section III /Annex III.12.3.3.1.b.
[20] Technical dossier/Section III /Annex III.12.3.3.1.d.
[21] Technical dossier/ Supplementary information November 12/Supplementary Data File No 1.
[22] Technical dossier/ Supplementary information November 12/Supplementary Data File No 2.

carried out by the non-parametric Wilcoxon Kruskal–Wallis (rank sum) test with chi-square approximation.

Table 2: Characteristics of the forage samples used in the ensiling studies

Study No	Test material	Dry matter content (%)	Water-soluble carbohydrate content (% fresh matter)
1[23]	First-cut permanent pasture grass (wilted for six hours)	28.3	6.8
2[24]	First-cut permanent pasture grass (wilted for six hours)	27.3	7.9
3[25]	Red clover (wilted)	42.9	3.2
4[26]	First-cut grass (wilted)	29.9	3.8
5[27]	Second-cut grass (wilted for 12 hours)	29.9	2.2
6[28]	Third-cut grass (wilted two days)	34.5	3.0
7[29]	Red clover (*Trifolium pratense*) (wilted)	25.7	2.3
8[30]	Red clover (*Trifolium pratense*) (wilted)	27.9	2.3
9[31]	Lucerne (*Medicago sativa*) (wilted)	41.2	2.8
10[32]	Bird's foot trefoil (*Lotus corniculatus*) (wilted)	29.2	2.0
11[33]	Lucerne (*Medicago sativa*) (unwilted)	21.7	1.4
12[34]	Permanent pasture grass (unwilted)	17.4	4.8
13[35]	Permanent pasture grass (wilted for 48 hours)	32.0	6.8
14[36]	Whole-crop barley	30.0	3.0
15[37]	Whole-crop wheat	32.0	3.6
16[38]	Whole-crop wheat	36.3	4.3
17[39]	Second-cut grass (wilted)	26.1	4.0
18[40]	Maize	30.3	3.4
19[41]	Maize	30.6	2.6
20[42]	Lucerne (wilted)	54.0	3.0

[23] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 1/Annex_2.
[24] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 1/Annex_3.
[25] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 1/Annex_4.
[26] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 1/Annex_5.
[27] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 1/Annex_6.
[28] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 1/Annex_7.
[29] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 1/Annex_8.
[30] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 1/Annex_9.
[31] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 1/Annex_10.
[32] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 1/Annex_11.
[33] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 1/Annex_12.
[34] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 2/Annex_2.
[35] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 2/Annex_3.
[36] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 2/Annex_4.
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[40] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 2/Annex_8.
[41] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 2/Annex_9.
[42] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 2/Annex_10.

Table 3: Summary of the analysis of ensiled material recovered at the end of the experiment with *Lactobacillus plantarum* NCIMB 40027 applied at the maximum recommended dose

Study No	Treatment (CFU/kg)	Dry matter loss (%)	pH	Lactic acid (% DM)	Acetic acid (% DM)	Ethanol (% DM)	Ammonia-N (% total N)[1]
1[43]	0	19.6	4.8	3.5	2.8	4.8	7.8
	1×10^9	9.5*	3.9*	10.1*	<0.1*	4.1	2.0*
2[44]	0	26.4	4.4	5.5	3.5	3.7	11.1
	1×10^9	19.6*	3.8*	9.5*	0.5*	3.7	2.9*
3[45]	0	5.0	5.1	2.3	1.2	0.9	6.8
	1×10^9	4.1*	4.1*	7.7*	1.0*	0.4*	2.7*
4[46]	0	–	4.8	4.3	1.1	1.5	21.9
	1×10^9	–	3.8*	11.0*	0.7*	0.7*	12.5*
5[47]	0	13.1	4.4	2.5	0.5	1.0	8.0
	1×10^9	10.6*	4.0*	4.7*	0.4*	1.1*	4.7*
6[48]	0	–	4.4	5.1	1.0	–	22.4
	1×10^9	–	4.2*	5.9	0.6*	–	12.4*
7[49]	0	10.1	5.3	4.6	2.6	1.9	21.5
	1×10^9	5.3*	4.2*	11.0*	1.6*	0.8*	2.1*
8[50]	0	11.4	5.8	0.7	1.4	2.4	27.1
	1×10^9	4.4*	3.9*	7.9*	1.3	0.9*	2.0*
9[51]	0	5.4	4.7	7.4	2.8	0.7	9.6
	1×10^9	4.2*	4.6*	7.2	2.1*	0.4*	6.5*
10[52]	0	3.9	4.5	5.7	1.3	0.6	6.5
	1×10^9	4.1*	4.0*	11.0*	1.5	0.6	3.4*
11[53]	0	3.7	4.6	5.2	6.1	0.8	14.1
	1×10^9	4.6	4.4*	8.2*	4.6	0.8	12.7*

*Significantly different from control at $P \leq 0.05$.

[43] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 1/Annex_2.
[44] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 1/Annex_3.
[45] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 1/Annex_4.
[46] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 1/Annex_5.
[47] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 1/Annex_6.
[48] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 1/Annex_7.
[49] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 1/Annex_8.
[50] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 1/Annex_9.
[51] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 1/Annex_10.
[52] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 1/Annex_11.
[53] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 1/Annex_12.

Table 4: Summary of the analysis of ensiled material recovered at the end of the experiment with *Lactobacillus plantarum* NCIMB 40027 applied at the minimum recommended dose

Study No	Treatment (CFU/kg)	Dry matter loss (%)	pH	Lactic acid (% DM)	Acetic acid (% DM)	Ethanol (% DM)	Ammonia-N (% total N)[+]
12[54]	0	1.3	4.0	9.2	2.3	1.1	(2.4)
	1×10^8	1.1	3.9*	11.6*	1.9*	1.2	(2.0)
13[55]	0	9.0	3.9	8.7	0.3	–	8.7
	1×10^8	9.2	3.7*	10.3*	0.3	–	6.4*
14[56]	0	1.5	4.0	7.5	1.7	0.9	6.6
	1×10^8	0.6*	3.9*	7.3	1.0*	0.5*	5.6*
15[57]	0	–	4.0	6.8	0.7	0.4	–
	1×10^8	–	3.8*	7.7*	0.3	0.7	–
16[58]	0	1.4	4.3	3.3	–	–	12.5
	1×10^8	0.5	3.9*	4.3*	–	–	6.2*
17[59]	0	–	3.8	6.7	0.6	0.7	–
	1×10^8	–	3.7*	6.5	0.6	0.8*	–
18[60]	0	–	3.9	4.5	1.2	0.4	(0.9)
	1×10^8	–	3.7*	5.1*	0.7*	0.4	(0.6*)
19[61]	0	–	4.0	4.4	2.7	0.7	(1.0)
	1×10^8	–	3.7*	5.6*	1.0*	0.6	(0.7*)
20[62]	0	–	4.5	5.1	0.6	–	(1.3)
	1×10^8	–	4.3*	5.0	0.4*	–	(0.7*)

[+]Values of ammonia-N in brackets are percentage of silage dry matter.
*Significantly different from control at $P \leq 0.05$.

Dry matter losses were reduced in seven of the nine studies in which the additive was applied at 1×10^9 CFU/kg silage (Table 3). Silage pH was significantly decreased by the addition of *L. plantarum* NCIMB 40027 in all 20 studies (Tables 3 and 4). Lactic acid content was significantly increased compared with control silos in 9 of the 11 studies in which *L. plantarum* was applied at 1×10^9 CFU/kg silage (Table 3) and in six of the nine studies in which the application rate of *L. plantarum* was 1×10^8 CFU/kg silage (Table 4). Acetic acid was significantly decreased in 13 out of 20 studies. Ammonia-N (as % of total N) was reduced in all the studies in which *L. plantarum* was added at 1×10^9 CFU/kg silage (Table 3) and in six out of seven studies with a rate of application of 1×10^8 CFU/kg silage (Table 4).

L. plantarum NCIMB 40027 has the potential to improve the production of silage by increasing lactic acid content and the preservation of dry matter, by reducing the pH and by moderately reducing the loss of protein, as determined by the ammonia-N content. This was demonstrated in a range of easy and moderately difficult to ensile forage materials at a minimum concentration of 1×10^8 CFU/kg fresh material and also in difficult to ensile forage materials at the addition rate of 1×10^9 CFU/kg fresh material.

[54] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 2/Annex_2.
[55] Technical dossier/Supplementary information November 12 /Annexes Supplementary Data File No 2/Annex_3.
[56] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 2/Annex_4.
[57] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 2/Annex_5.
[58] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 2/Annex_6.
[59] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 2/Annex_7.
[60] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 2/Annex_8.
[61] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 2/Annex_9.
[62] Technical dossier/Supplementary information November 12/Annexes Supplementary Data File No 2/Annex_10.

CONCLUSIONS

As the identity of the strain of *Lactobacillus plantarum* NCIMB 40027 has been established and no antibiotic resistance of concern detected, following the QPS approach the use of this strain in the production of silage is considered safe for target species, for consumers of products from animals fed treated silage and for the environment.

The active agent is not an eye or skin irritant or skin sensitiser. Given the proteinaceous nature of the active agent and the high dusting potential of the product tested, the FEEDAP Panel considers it prudent to treat this additive as a respiratory sensitiser.

L. plantarum NCIMB 40027 has the potential to improve the production of silage by increasing lactic acid content and the preservation of dry matter, by reducing the pH and by moderately reducing the loss of protein, as determined by the ammonia-N content. This was demonstrated in a range of easy and moderately difficult to ensile forage materials at a minimum concentration of 1×10^8 CFU/kg fresh material and also in difficult to ensile forage materials at the addition rate of 1×10^9 CFU/kg fresh material.

DOCUMENTATION PROVIDED TO EFSA

1. *Lactobacillus plantarum* (NCIMB 40027). November 2010. Submitted by Ecosyl Products Ltd.
2. *Lactobacillus plantarum* (NCIMB 40027). Supplementary information. November 2012. Submitted by Volac International Ltd.
3. *Lactobacillus plantarum* (NCIMB 40027). Supplementary information. January 2013. Submitted by Volac International Ltd.
4. Evaluation report of the European Union Reference Laboratory for Feed Additives on the methods of analysis for *Lactobacillus plantarum* (NCIMB 40027).
5. 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.

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2012a. Scientific Opinion on the maintenance of the list of QPS biological agents intentionally added to food and feed (2012 update). EFSA Journal 2012;10(12):3020, 84 pp. doi:10.2903/j.efsa.2012.3020

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012b. 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

APPENDIX

Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for *Lactobacillus plantarum* (NCIMB 40027)[63]

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.[64] 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.

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

[64] 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