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Microbiological and chemical profile of sugar cane silage fermentation inoculated with wild strains of lactic acid bacteria

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ABSTRACT

During sugar cane ensilage an intense growth of yeasts can result in high dry matter (DM) loss and reduction in the quality of the feed. The aim of this study was to evaluate the fermentation profile of sugar cane (*Saccharum* spp.) silage inoculated with new strains of lactic acid bacteria (LAB) screened for this forage silage. Fourteen wild LAB strains were evaluated, biochemically (API 50 CHL, BioMérieux) characterized, and identified by sequencing of 16S rDNA. The wild isolates were identified as *Lactobacillus plantarum*, *Lactobacillus brevis*, and *Lactobacillus hilgardii*. Different fermentation profiles were observed among strains of the same species. The silages inoculated with *L. plantarum* species showed the highest yeast population (5.97 log CFU/g silage), ethanol concentration (137 g kg/silage) and DM loss (20.6%) ($P<0.01$), therefore, they were not beneficial for sugar cane silage. The silages inoculated with *L. brevis* UFLA SIL17 and UFLA SIL24 and *L. hilgardii* UFLA SIL51 and UFLA SIL52 strains showed smaller DM loss (12.2%) and NDF-NDF content (573 g/kg silage) ($P<0.01$). When compared with these inoculated silages, the silages inoculated with *L. hilgardii* UFLA SIL51 and UFLA SIL52 strains resulted in 57% and 94% more acetic acid and 1,2-propanediol respectively. Obligatory heterofermentative strains showed better silage quality. *L. hilgardii* (UFLA SIL51 and UFLA SIL52) strains show promise for use in sugar cane silage.

1. Introduction

Many factors contribute to the success of the natural fermentation of carbohydrate-rich foods such as sugar cane (*Saccharum* spp.), a forage mainly used in animal feed in subtropical and tropical regions. One of the main difficulties with the conservation of this forage as silage is the high dry matter (DM) loss (Kung and Stanley, 1982) caused primarily by growth of yeasts (Pedroso et al., 2005). The metabolic activity of lactic acid bacteria (LAB) plays a key role during the fermentation of the silage and is chiefly responsible for overall silage quality (Ávila et al., 2009; Dunière et al., 2013).

The use of microbiological additives promotes the dominance of inoculated strains and controls the growth of undesirable microorganisms (Saarisalo et al., 2007), resulting in a faster decrease in pH value. The compatibility between the forage and

Abbreviations: DM, dry matter; LAB, lactic acid bacteria; rDNA, ribossomic DNA; PVC, polyvinyl chloride; PCR, polymerase chain reaction; FM, fresh matter; NDF–NDF, neutral detergent fiber; WSC, water soluble carbohydrates; HPLC, high-performance liquid chromatography; CFU, colony-forming units.

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the inoculant strain is a determining factor for the success of a microbial additive (Ávila et al., 2009; Yang et al., 2010). Wild strains isolated from a forage crop may show results superior to commercial strains when inoculated in the same culture from which they were isolated (Ávila et al., 2009). From these results, the prospection of silage strains has great biotechnological potential for development of new products. Despite the great potential for LAB use as starter cultures in silage, the results of using these inoculants have been controversial depending on the substrate used and the parameters evaluated (Kleinschmit and Kung, 2006). Therefore, it is important to study the action of inoculants during the fermentation process.

The objective of the present study was to evaluate strains of LAB isolated from sugar cane silage during ensilage of this forage, with particular interest in the species capable of reducing DM loss, thus maintaining the nutritive value of forage. The chemical and microbiological composition and silage fermentation characteristics were also studied.

2. Materials and methods

2.1. Biochemical and molecular characterization of the utilized additives

The strains were isolated from sugar cane silage and selected through laboratory tests (Ávila et al., 2014). They belong to a culture collection of the Laboratory of Microbial Physiology and Genetics at Department of Biology/Federal University of Lavras (UFLA). Characterized strains of the genus *Lactobacillus* were evaluated for the production of metabolites (Ávila et al., 2014), and the strains with the best production of lactic acid, acetic acid, and propionic acid were selected for evaluation in experimental silos.

The strains were biochemically characterized by sugar fermentation standards using API 50 CHL kits (BioMérieux). Gas production was evaluated with a Durham tube in MRS broth (De Man, Rogosa, & Sharpe, Oxoid CM361, Basingstoke, Hampshire, England) according Chao et al. (2008). These strains were also identified by 16S rDNA sequencing. Each isolate was grown in MRS agar plates for 24 h at 30 °C and collected with a sterile pipette tip, then resuspended in 40 μl of PCR buffer. To achieve the DNA template, the suspension was heated for 10 min at 95 °C, and 2 μl was used in PCR experiments to amplify the full-length 16S region. An approximately 1500-bp fragment of the 16S rDNA was amplified using the forward primer 27f (5′AGAGTTTGATCCTGGCTCAG3′) and the reverse primer 1512r (5′ACGGCTACCTTGTTACGACT3′). The PCR products were sequenced using an ABI3730 XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA). The sequences were compared to the GenBank database using the BLAST algorithm (National Center for Biotechnology Information, Maryland, USA).

2.2. Forage and ensilage conditions

The silages were made on three different days; on each day one replication of each treatment (different strains and different times of silage fermentation) were made. In each day, fresh-cut sugar cane aged approximately 12 months was manually harvested and chopped (PP-47, Pinheiro, Itapira, SP, Brazil). Experimental PVC (polyvinyl chloride) mini-silos, sized 10 cm in diameter and 60 cm in length, were used. The experimental silos were sealed with tight lids containing Bunsen valves for gas release. Three silos were prepared for each evaluation day (12, 30, 61, and 126) with one of the 14 strains plus a control treatment (without inoculants). The new inoculants were pre-cultured in the laboratory and were enumerated on MRS agar on the ensilage day. The inoculums concentration included 1.8×10^6 CFU/g fresh matter (FM). The inoculants were mixed with pure distilled water and sprayed in the forage, resulting in an application volume of 14.3 L/ton. The same volume of pure distilled water was added to the control treatment. A separate sprayer was used for each treatment to avoid cross-contamination. Each silo was packed with 3 kg of fresh forage to achieve a packing density of approximately 638 kg/m^3 of FM, and the weight of empty and full silos was recorded. The silos were sealed, stored at room temperature (25 ± 1.5 °C), and protected from sunlight and rain. After 12, 30, 61, and 126 days of ensilage, the full silos were weighed and opened. The total loss of DM was calculated using weights of the DM contents of fresh forage and silage.

2.3. Analytical procedures

To obtain the aqueous extract, a 25-g sample of fresh forage or sugar cane silage was blended in 225 mL of 0.1% sterile peptone water and homogenized in an orbital shaker for 20 min. The pH of each sample was then determined (DIGIMED® DM 20 Potentiometer, Digicrom Instrumentos, SP, Brazil). Aqueous extracts (2 mL) were acidified with 10 μL of 50% (vol/vol) H_2SO_4 and frozen prior to analysis of fermentation end products. The acidified aqueous extracts were analyzed for ethanol, acetic, butyric, propionic, and lactic acids, and 1,2-propanediol by high-performance liquid chromatography according to the method described by Carvalho et al. (2012). The DM contents of each sample were determined using a forced-draft oven at 55 °C for 72 h. Dried samples were ground in a Wiley-type grinder through a 1-mm screen. DM at 105 °C was determined by drying 1 g for 24 h. Neutral detergent fiber (NDF-NDF) was analyzed without a heat stable amylase and using sodium sulfite, by means of an ANKOM 200 Fiber Analyzer (ANKOM Technology Corp., Macedon, NY, USA). NDF-NDF was expressed inclusive of residual ash (Van Soest et al., 1991). Water soluble carbohydrates (WSC) were analyzed using the phenol method (Dubois et al., 1956).

Table 1
Properties and identification of the lactic acid bacteria (LAB) strains evaluated.

Strain	Involved acid [a]	Gas production[b]	Biochemical identification	Molecular identification[c]
UFLA SIL19	Lactic	–	*L. plantarum* 99.9%	*L. plantarum* 98% (FJ669130.1)[d]
UFLA SIL32	Lactic	–	*L. plantarum* 99.9%	*L. plantarum* 99% (HM218291.1)
UFLA SIL33	Lactic	+	*L. brevis* 96.3%	*L. brevis* 98% (FJ227316.1)
UFLA SIL34	Lactic	–	*L. plantarum* 99.9%	*L. plantarum* 98% (HM218291.1)
UFLA SIL17	Acetic	+	*L. brevis* 99.8%	*L. brevis* 97% (FJ532364.1)
UFLA SIL24	Acetic	+	*L. brevis* 99.9%	*L. brevis* 99% (FJ227316.1)
UFLA SIL25	Acetic	+	*L. brevis* 96.3%	*L. brevis* 98% (FJ227316.1)
UFLA SIL27	Acetic	+	*L. brevis* 96.3%	*L. brevis* 98% (FJ227316.1)
UFLA SIL35	Acetic	–	*L. plantarum* 99.9%	*L. plantarum* 98% (HM218291.1)
UFLA SIL41	Propionic	–	*L. plantarum* 99.9%	*L. plantarum* 99% (HM218291.1)
UFLA SIL42	Propionic	–	*L. plantarum* 99.6%	*L. plantarum* 99% (HM218291.1)
UFLA SIL46	Propionic	–	*L. plantarum* 99.6%	*L. plantarum* 99% (HM218291.1)
UFLA SIL51	Propionic	+	*L. buchneri* 99.8%	*L. hilgardii* 99% (HM217953.1)
UFLA SIL52	Propionic	+	*L. buchneri* 99.8%	*L. hilgardii* 99% (HM217953.1)

[a] Involved acid in the screening process based on the production of metabolites in sugar cane broth (Ávila et al., 2014).
[b] From MRS broth.
[c] Sequencing of 16S rDNA.
[d] The number in parentheses refers to the access code in Gen-Bank.

2.4. Microbiological analyses

The other portion of aqueous extracts was used for the enumeration of microorganisms. Sequential tenfold dilutions were prepared to quantify the microbial groups. Yeasts and filamentous fungi were enumerated on Dichloran Rose Bengal Chloramphenicol Medium (DRBC, Difco, Becton Dickinson, Sparks, MD, USA). The plates were incubated at 28 °C for 72 h. Yeasts were distinguished from filamentous fungi by colony appearance and cell morphology. For enumeration of LAB, pour plating onto DeMan Rogosa Sharpe agar plus nystatin (4 mL/L) was used. The plates were incubated at 30 °C for 72 h. Colonies were counted on plates containing a minimum of 30 and a maximum of 300 colony-forming units (CFUs).

2.5. Statistical analysis

The experiment was carried out in randomized blocks; each block corresponded to a day of silage production. The treatments were assigned in a factorial arrangement consisting of 15 combinations (14 LAB strains and a control without inoculant) and 4 silage fermentation periods (12, 30, 61, and 126 days). Twelve silos (3 replications and 4 opening days) were prepared for each microbial strain, totaling 180 experimental units. The data were analyzed by SISVAR®, by a model containing the fixed effects of blocks, inoculants, days of ensilage, and the interactions between the inoculants and the days of ensilage. The means were compared using a Scott-Knott test.

The principal component analyses (PCA) were performed using the software XLSTAT 7.5.2 (Addinsoft, New York, NY, USA) for grouping data of the fermentation products, DM loss, DM, and NDF-NDF with evaluated strains. The data utilized in the PCA analysis were related to the average results of all ensiling periods evaluated.

3. Results

3.1. Biochemical characteristics and molecular identification of strains

The characteristics, identification, and API 50 CHL (BioMérieux) fermentation patterns of selected strains are shown in Tables 1 and 2. The *Lactobacillus brevis* (UFLA SIL33, UFLA SIL17, UFLA SIL24, UFLA SIL25, and UFLA SIL27) and *Lactobacillus hilgardii* (UFLA SIL51 and UFLA SIL52) strains produced gas when cultivated in MRS broth. All strains of lactic acid bacteria were able to ferment L-arabinose, D-ribose, D-glucose, D-fructose, and D-maltose. Among the strains identified as *Lactobacillus plantarum*, UFLA SIL19, UFLA SIL32, UFLA SIL34, UFLA SIL35, and UFLA SIL41 fermented a higher number of carbohydrates (Table 2). Saccharose, the main carbohydrate present in the sugar cane, was one of the fermentable carbohydrates by evaluated isolates. Strains UFLA SIL42 and UFLA SIL46, both identified as *L. plantarum*, were able to ferment 21 carbohydrates, but not saccharose, methyl-αD-mannopyranoside, or D-rafinose.

L. brevis strains also showed differences in the use of carbohydrates, but none was able to ferment D-saccharose (Table 2). Strains UFLA SIL51 and UFLA SIL52 were biochemically identified as *Lactobacillus buchneri* (99.8%) and were able to ferment eleven different carbohydrates, including D-saccharose (Table 2). The 14 strains were also identified by the sequencing of the 16S region of the rDNA to confirm the biochemical identification. The sequencing results identified the UFLA SIL51 and UFLA SIL52 strains as *L. hilgardii* (98%). In the identification of the other 12 strains, the sequencing results confirmed the biochemical identification.

Table 2
Fermentation patterns (evaluated using API 50 CHL strips; BioMerieux) of lactic acid bacteria strains evaluated.

Item	UFLA SIL19♦	UFLA SIL32♦	UFLA SIL33‡	UFLA SIL34♦	UFLA SIL17‡	UFLA SIL24‡	UFLA SIL25‡	UFLA SIL27‡	UFLA SIL35♦	UFLA SIL41♦	UFLA SIL42♦	UFLA SIL46♦	UFLA SIL51*	UFLA SIL52*
D-Galactose	+[a]	+	+	+	+	+	+	+	+	+	+	+	–	–
D-Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Manose	+	+	–	+	–	–	–	–	+	+	+	+	–	–
L-Rhamnose	w	w	–	w	–	–	–	–	–	w	w	w	–	–
D-Manitol	+	+	–	+	+	+	–	–	+	+	+	+	–	–
D-Sorbitol	+	+	–	+	–	–	–	–	+	+	+	+	–	–
Methyl-αD-mannopyranoside	+	+	–	+	–	–	–	–	+	+	–	–	–	–
Methyl-αD-glucopyranoside	–	–	w	–	+	+	w	–	–	–	+	+	–	–
N-acetylglucosamine	+	+	w	+	+	+	w	w	+	+	+	+	–	–
Amygdalin	+	+	–	+	–	–	–	–	+	+	+	+	–	–
Arbutin	+	+	–	+	–	–	–	–	+	+	+	+	–	–
Esculin ferric citrate	+	+	w	+	–	–	w	–	+	+	+	+	–	–
Salicin	+	+	–	+	–	–	–	–	+	+	+	+	–	–
D-Celobiose	+	+	–	+	–	–	–	–	+	+	+	+	–	–
D-Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Lactose	+	+	–	+	–	–	–	–	+	+	+	+	–	–
D-Melibiose	+	+	+	+	+	+	+	+	+	+	+	+	–	–
Sucrose	+	+	–	+	–	–	–	–	+	+	–	–	+	+
D-Trehalose	+	+	–	+	–	–	–	–	+	+	+	+	–	–
D-Melezitose	+	+	–	+	–	–	–	–	+	+	+	+	+	+
D-Rafinose	+	+	–	+	–	–	–	–	+	+	–	–	+	+
Gentiobiose	+	w	–	+	–	–	–	–	w	+	+	+	–	–
D-Turanose	+	+	–	+	–	–	–	–	+	+	–	–	–	–
Potassium gluconate	w	w	w	w	w	+	w	w	w	w	–	–	+	+
Potassium 2-ketogluconate	–	–	–	–	–	+	–	–	–	–	–	–	–	–
Potassium 5-ketogluconate	–	–	w	–	w	+	w	w	–	–	–	–	+	+

All strains gave negative results for glycerol, erythritol, D-arabinose, L-xylose, D-adonitol, L-sorbose, dulcitol, inositol, inulin, starch, glycogen, xylitol, D-lyxose, D-tagatose, D-frucose, L-frucose, D-arabitol, L-arabitol.

[a] + = positive reaction; – = negative reaction; and w = weakly positive reaction.

♦ *Lactobacillus plantarum*.

‡ *Lactobacillus brevis*.

* *Lactobacillus hilgardii*.

Table 3
Chemical and microbial composition of fresh whole-plant of sugar cane.

Item	Mean/standard deviation
Lactic acid bacteria (log CFU/g fresh forage)	7.51[a] ± 0.617
Yeasts (log CFU/g fresh forage)	5.72 ± 0.186
Filamentous fungi (log CFU/g fresh forage)	5.07 ± 0.153
pH	5.75 ± 0.046
Dry matter (g/kg)	282 ± 1.17
Neutral detergent fiber (g/kg DM)	480 ± 1.78
Water soluble carbohydrates (g/kg DM)	247 ± 0.92
Density (kg/m^3)	634.1 ± 19.9

[a] Each mean was obtained in nine replicates.

3.2. Fresh forage

The characteristics of the sugar cane prior to the ensilage are shown in Table 3. LAB, filamentous fungi, and yeasts were observed in the forage at population levels of 7.51, 5.07, and 5.72 log CFU/g forage, respectively. The average density obtained in the mini-silos after sealing was 634 kg/m^3 of fresh forage.

3.3. Chemical composition of the silages and fermentative loss

The addition of different strains influenced the concentrations of DM and NDF-NDF and the losses of DM ($P<0.01$) (Table 4). The silages inoculated with the *L. hilgardii* UFLA SIL51 and UFLA SIL52 and *L. brevis* UFLA SIL24 and UFLA SIL17 strains showed the lowest DM losses. These silages also showed the highest concentrations of DM and the lowest concentrations of NDF-NDF. These variables changed during the time of fermentation ($P<0.01$). The concentration of DM decreased from 277 to 250 g/kg, while those of the NDF-NDF and the losses of DM increased from 547 to 633 g NDF-NDF/kg DM and from 7.9 to 21.7 % of DM loss from fermentation days 12 to 61. On day 61, the concentrations of these variables stabilized: 254 g DM/kg; 625 g NDF-NDF/kg DM; 22.3 % of DM loss.

No significant difference in pH values of the silages was observed between the inoculated strains ($P=0.98$) (Table 5). Thus, the modifications that occurred throughout the fermentation process were similar between the silages, and the pH was stable until day 61. From fermentation days 61 to 126, an increase in pH was observed (Table 5).

When the WSC concentration was examined, a significant interaction between the factor strains and days of ensilage ($P<0.01$) was observed (Table 5). At day 12, the lowest concentrations of WSC were observed in the silages treated with the *L. hilgardii* UFLA SIL51 and UFLA SIL52 strains, and the highest concentration was observed in the silage inoculated with the

Table 4
Dry matter (DM), neutral detergent fiber (NDF–NDF) and DM loss (DML) (DM basis) of sugar cane silages with novel inoculants.

Inoculants	DM	NDF	DML
	g/kg	g/kg DM	% of ensiled DM
Control	263 b	571 b	16.4 c
UFLA SIL19♦	250 c	623 a	21.3 a
UFLA SIL32♦	250 c	631 a	21.2 a
UFLA SIL33‡	270 a	576 b	14.6 c
UFLA SIL34♦	248 c	651 a	22.9 a
UFLA SIL17‡	270 a	556 b	13.1 d
UFLA SIL24‡	275 a	569 b	12.5 d
UFLA SIL25‡	267 b	565 b	14.2 c
UFLA SIL27‡	271 a	570 b	13.9 c
UFLA SIL35♦	251 c	638 a	21.4 a
UFLA SIL41♦	250 c	639 a	20.5 a
UFLA SIL42♦	255 c	630 a	18.4 b
UFLA SIL46♦	253 c	642 a	18.6 b
UFLA SIL51*	274 a	578 b	11.4 d
UFLA SIL52*	269 a	590 b	11.8 d
SEM[a]	2.21	7.81	0.89
P-value			
Inoculants (I)	<0.01	<0.01	<0.01
Days of ensilage (DE)	<0.01	<0.01	<0.01
I × DE	0.37	0.58	0.77

a–d Means from triplicate samples. For each column, means values with different small letters are significant at $P<0.05$ by Scott–Knott test.
[a] Standard error of the means.
♦ *Lactobacillus plantarum*.
‡ *Lactobacillus brevis*.
* *Lactobacillus hilgardii*.

Table 5
Effects of inoculation with different strains and of different days of ensilage on the concentrations of water soluble carbohydrate, ethanol, lactic acid and pH in sugar cane silages.

Days of ensilage	Control[a]	UFLA SIL19♦	UFLA SIL32♦	UFLA SIL33‡	UFLA SIL34♦	UFLA SIL17‡	UFLA SIL24‡	UFLA SIL25‡	UFLA SIL27‡	UFLA SIL35♦	UFLA SIL41♦	UFLA SIL42♦	UFLA SIL46♦	UFLA SIL51*	UFLA SIL52*
Water soluble carbohydrate (g/kg DM)															
12	136.2 bA[1]	171.4 aA	148.5 bA	110.3 cA	128.8 bA	105.3 cA	109.6 cA	117.3 cA	113.1 cA	144.5 bA	138.7 bA	134.5 bA	134.5 bA	75.5 dA	66.7 dA
30	78.1 aB	88.1 aB	72.8 aB	33.4 bB	69.5 aB	49.8 bB	57.4 aB	34.9 bB	46.7 bB	73.1 aB	60.2 aB	56.6 aB	68.8 aB	41.2 bB	38.2 bB
61	25.3 aC	33.6 aC	32.3 aC	23.2 aB	21.8 aC	25.5 aC	24.5 aC	30.8 aB	24.0 aC	24.6 aC	31.6 aC	25.4 aC	21.2 aC	23.4 aB	21.1 aB
126	13.1 aC	14.1 aC	14.6 aC	13.1 aB	14.2 aC	13.7 aC	12.0 aC	13.3 aB	13.8 aC	13.5 aC	13.8 aC	15.9 aC	12.5 aC	20.9 aB	19.4 aB
Level of significance				Inoculants <0.01			Days of ensilage <0.01			Inoculants x Days of ensilage <0.01				SEM[b] 7.56	
Ethanol (g/kg DM)															
12	26.7 bB	48.9 aC	51.4 aC	19.3 bB	73.8 aC	23.2 aB	17.0 bB	11.2 bB	18.1 bB	61.5 aC	45.2 aC	55.2 aB	54.3 aC	27.9 aA	35.7 aA
30	46.6 dB	90.1 cB	113.3 bB	73.5 cA	164.5 aB	35.5 dB	46.1 dB	85.3 cA	70.1 cA	131.4 bB	148.0 aB	85.7 cB	117.4 bB	38.6 dA	38.2 dA
61	137.1 bA	189.3 aA	210.1 aA	87.2 cA	217.8 aA	76.2 cA	94.4 cA	88.9 cA	102.7 cA	185.8 aA	208.3 aA	187.5 aA	180.4 aA	39.9 dA	44.4 dA
126	104.2 cA	174.0 aA	171.5 aA	72.3 cA	207.1 aA	85.1 cA	80.2 cA	77.3 cA	77.2 cA	200.5 aA	137.6 bB	180.6 aA	144.1 bB	32.4 dA	36.0 dA
Level of significance				Inoculants <0.01			Days of ensilage <0.01			Inoculants x Days of ensilage <0.01				SEM 14.14	
Lactic acid (g/kg DM)															
12	17.2 cB	42.6 aB	41.8 aA	30.1 bA	40.8 aA	28.4 bB	28.2 bB	27.5 bA	29.8 bA	34.1 bB	40.1 aA	33.8 bB	37.1 aB	31.6 bA	32.0 bA
30	25.2 bB	41.4 aB	23.9 bB	28.5 bA	38.3 aA	25.4 bB	27.2 bB	27.3 bA	29.0 bA	42.0 aA	42.6 aA	38.3 aB	32.5 bB	32.4 bA	31.0 bA
61	46.6 aA	55.3 aA	51.8 aA	34.5 bA	48.5 aA	35.5 bA	36.4 bA	36.8 bA	35.7 bA	43.7 aA	47.9 aA	48.1 aA	43.7 aA	34.6 bA	31.0 bA
126	48.6 aA	47.1 aB	43.8 aA	29.6 bA	45.8 aA	36.9 bA	37.5 bA	33.9 bA	34.5 bA	48.1 aA	42.3 aA	54.7 aA	46.0 aA	30.0 bA	27.6 bA
Level of significance				Inoculants <0.01			Days of ensilage <0.01			Inoculants x Days of ensilage <0.01				SEM 3.30	
pH															
12	3.84 aA	3.56 aA	3.54 aA	3.53 aA	3.57 aA	3.59 aA	3.58 aA	3.59 aA	3.55 aA	3.55 aB	3.55 aA	3.67 aA	3.62 aB	3.56 aA	3.52 aB
30	3.63 aA	3.59 aA	3.64 aA	3.65 aA	3.64 aA	3.62 aA	3.59 aA	3.66 aA	3.66 aA	3.62 aB	3.64 aA	3.63 aA	3.63 aB	3.59 aA	3.64 aB
61	3.60 aA	3.60 aA	3.61 aA	3.62 aA	3.64 aA	3.61 aA	3.62 aA	3.61 aA	3.63 aA	3.64 aB	3.58 aA	3.61 aA	3.62 aB	3.61 aA	3.66 aB
126	3.75 aA	3.73 aA	3.76 aA	3.76 aA	3.78 aA	3.75 aA	3.77 aA	3.70 aA	3.75 aA	3.83 aA	3.72 aA	3.82 aA	3.86 aA	3.80 aA	3.84 aA
Level of significance				Inoculants 0.98			Days of ensilage <0.01			Inoculants x Days of ensilage 0.99				SEM 0.077	

a–d For each row, mean values with different lowercase letters are significant at P<0.05 by Scott–Knott test.
A–C For each column, mean values with different capital letters are significant at P<0.05 by Scott–Knott test.
[a] Control: without inoculants.
[b] Standard error of the means of two-way interactions (inoculants and days of ensilage).
♦ *Lactobacillus plantarum.*
‡ *Lactobacillus brevis.*
* *Lactobacillus hilgardii.*

L. plantarum UFLA SIL19 strain (Table 5). The highest average losses of WSC occurred from 12 to 30 days (52.8%). There was no difference between the WSC concentrations of the silages from fermentation days 61 and 126 (Table 5).

There was an interaction (P<0.01) between the inoculated strains and the days of ensilage in relation to ethanol, 1,2-propanediol, and lactic, acetic, propionic, and butyric acid concentrations. The exception was the *L. hilgardii* UFLA SIL51 and UFLA SIL52 strains, which showed low rates of ethanol throughout the fermentation. The control and silages treated with the other strains showed an increase in the ethanol concentration during the ensilage period. Ethanol concentration showed the most outstanding differences between silages after day 30 of fermentation. On day 126 of ensilage, the UFLA SIL51 and UFLA SIL52 strains showed the lowest ethanol concentrations.

The silages inoculated with *L. plantarum* strains produced higher quantities of lactic acid than the silages inoculated with *L. brevis* and *L. hilgardii* strains (Table 5). In the silages inoculated with strains UFLA SIL33, UFLA SIL34, UFLA SIL25, UFLA SIL27, and UFLA SIL41, day 12 of fermentation showed a peak in lactic acid concentration, which did not further increase until day 120. However, for the other silages, the lactic acid concentration increased at different rates.

With the exception of the silages treated with the *L. plantarum* UFLA SIL34, UFLA SIL27, UFLA SIL35, UFLA SIL42, UFLA SIL46, and *L. brevis* UFLA SIL27 strains, the presence of acetic acid in the silage increased, showing the highest values after day 126 of ensilage (Table 6). Considering all the periods evaluated, the highest average of acetic acid concentrations were observed in the silages inoculated with the *L. hilgardii* UFLA SIL51 and UFLA SIL52 strains, followed by the silages inoculated with *L. brevis* UFLA SIL33, UFLA SIL25, UFLA SIL24, UFLA SIL27, and UFLA SIL17 strains.

The concentrations of propionic acid were variable throughout the ensilage period for each strain (Table 6). Analysis of the averages for all the evaluation periods, showed that the silages treated with the *L. plantarum* UFLA SIL41, UFLA SIL32, UFLA SIL35, UFLA SIL34, and with the *L. brevis* UFLA SIL17, UFLA SIL33, UFLA SIL25, and UFLA SIL27 strains obtained the highest concentrations of propionic acid (Table 6). In general, the presence of 1,2-propanediol in the silage was low, and there was no significant difference throughout the ensilage, except in the silages treated with the *L. hilgardii* UFLA SIL51 and UFLA SIL52 strains. In these silages, the concentration of the metabolite increased from day 30 of ensilage and was significantly higher (Table 6).

During the ensilage period, no increase was observed in the butyric acid concentration in the control silage or in the silages treated with the UFLA SIL19, UFLA SIL42, and UFLA SIL52 strains. The remaining silages showed increased butyric acid concentration. At 126 days of ensilage, butyric acid was not detected in the control silage or silage treated with the *L. plantarum* UFLA SIL19 strain.

3.4. Microbial population of the silages

There was a significant effect on LAB population by the interaction between the inoculants and number of ensilage days (P<0.01) (Table 7). At 30 and 61 days of ensilage, the greatest LAB populations were observed in the silages treated with the *L. hilgardii* UFLA SIL51 and UFLA SIL52 strains, followed by the control silage. The UFLA SIL51 and UFLA SIL52 strains showed a great decrease in LAB population compared to the remaining silage.

There was no interaction between days of ensilage and inoculated strains in the yeast populations (P=0.23). The addition of the new strains significantly modified the populations of yeasts in the silages (P<0.01). The silages inoculated with the *L. hilgardii* and *L. brevis* strains showed the lowest, while silages treated with *L. plantarum* strains showed the highest yeast populations (Table 7). During the fermentation period, the population of yeasts decreased from day 30 (P<0.01) (Table 7). Filamentous fungi were not observed in the silages.

3.5. Multivariate analyses

To examine the relationship between the DM loss, NDF-NDF, DM, fermentation products, and the strain inoculated in silage, the average of data from Tables 4–7 were subjected to principal component analysis (PCA). Fig. 1 shows the biplot of PCA. The first (PC 1) and second (PC 2) principal components explain 58.6 and 19.9% of the total variance, respectively.

The results plot (Fig. 1) showed the formation of two groups. One of the groups was located on the negative part of the PC 1 and included the silages inoculated with obligatory heterofermentative of *L. brevis* and *L. hilgardii* strains; the other group was closely related to the positive part of the axis, and included the silages treated with facultatively heterofermentative *L. plantarum* strains.

Component 2 allowed the differentiation of the silages inoculated with *L. brevis* strains from the silages inoculated with *L. hilgardii* strains. The obligatory heterofermentative strains were correlated with high concentrations of acetic acid, 1,2-propanediol, DM, and LAB populations. Within this group, the *L. hilgardii* UFLA SIL51 and UFLA SIL52 strains showed the highest 1,2-propanediol production. High concentrations of ethanol, NDF-NDF, lactic acid, and yeast populations were correlated with high DM loss; *L. plantarum* strains were also correlated with these variables (Fig. 1).

4. Discussion

Strains UFLA SIL19, UFLA SIL32, UFLA SIL34, UFLA SIL35, UFLA SIL41, UFLA SIL42, and UFLA SIL46 were identified as *L. plantarum*. UFLA SIL33, UFLA SIL17, UFLA SIL24, UFLA SIL25, and UFLA SIL27 were identified as *L. brevis*. These species are commonly found in silages and have been identified and used as inoculants in sugar cane silages (Ávila et al., 2009; Ávila

Table 6
Effects of inoculation with different strains and of different days of ensilage on the concentrations of acetic acid, propionic acid, 1,2 propanediol and butyric acid in sugar cane silages.

Days of ensilage	Control[a]	UFLA SIL19♦	UFLA SIL32♦	UFLA SIL33‡	UFLA SIL34♦	UFLA SIL17‡	UFLA SIL24‡	UFLA SIL25‡	UFLA SIL27‡	UFLA SIL35♦	UFLA SIL41♦	UFLA SIL42♦	UFLA SIL46♦	UFLA SIL51*	UFLA SIL52*
Acetic acid (g/kg DM)															
12	3.3 bB[1]	1.6 bB	1.6 bB	7.5 aB	1.8 bA	7.3 aB	6.6 aB	7.3 aB	7.4 aA	1.4 bA	1.4 bB	2.6 bA	2.1 bA	7.6 aB	8.4 aB
30	2.8 bB	2.0 bB	2.0 bB	9.0 aB	3.3 bA	5.0 bB	7.0 aB	11.5 aB	10.4 aA	2.2 bA	2.6 bB	2.6 bA	2.1 bA	12.8 aB	12.2 aB
61	9.7 bA	6.0 aB	3.9 cB	9.8 bB	4.1 cA	10. 5 bA	10.5 bB	10.6 bB	9.9 bA	3.3 cA	3.6 cB	8.9 bA	3.2 cA	19.7 aA	22.5 aA
126	10.9 bA	11.1 bA	9.2 cA	20.3 aA	7.3 cA	12.9 bA	20.2 aA	15.7 aA	13 bA	5.8 cA	13.3 bA	5.8 cA	6.9 cA	21.5 aA	20.6 aA
Level of significance			Inoculants			Days of ensilage				Inoculants x Days of ensilage				SEM[b]	
			<0.01			<0.01				<0.01				1.92	
Propionic acid (g/kg DM)															
12	4.0 aA	2.4 aA	4.3 aC	3.5 aB	5.1 aA	3.7 aB	3.4 aA	3.1 aB	3.4 aB	4.3 aB	4.4 aB	3.2 aA	3.8 aA	4.2 aA	4.5 aA
30	1.8 cA	2.5 cA	5.5 bB	6.5 aA	6.3 aA	4.8 bB	4.8 bA	6.9 aA	6.5 aA	6.2 aA	7.0 aA	2.8 cA	4.0 cA	4.6 bA	4.7 bA
61	3.1 cA	3.4 cA	7.6 aA	7.4 aA	5.4 bA	6.5 aA	3.4 cA	5.2 bA	5.2 bA	6.4 aA	6.6 aA	3.3 cA	3.5 cA	4.0 cA	4.1 cA
126	3.3 aA	2.7 aA	1.9 aD	1.8 aB	1.8 aB	1.4 aC	2.0 aA	1.9 aB	1.8 aB	1.8 aC	3.3 aB	2.9 aA	2.8 aA	2.2 aB	2.0 aB
Level of significance			Inoculants			Days of ensilage				Inoculants x Days of ensilage				SEM	
			<0.01			<0.01				<0.01				0.63	
1,2 propanediol (g/kg DM)															
12	0.5 aA	0.5 aA	0.3 aA	0.4 aA	0.5 aA	0.5 aA	0.4 aA	0.3 aA	0.4 aA	0.5 aA	0.5 aA	0.5 aA	0.5 aA	0.7 aD	1.1 aD
30	0.3 cA	0.5 cA	0.5 cA	0.5 cA	0.5 cA	0.4 cA	0.4 cA	0.5 cA	0.5 cA	0.5 cA	0.6 cA	0.6 cA	0.5 cA	14.5 bC	19.3 aC
61	1.7 cA	0.5 cA	0.6 cA	0.6 cA	0.6 cA	0.7 cA	0.5 cA	0.6 cA	0.6 cA	0.6 cA	0.7 cA	0.6 cA	0.6 cA	32.8 bA	39.9 aA
126	2.6 cA	0.5 cA	0.5 cA	0.6 cA	0.6 cA	0.7 cA	0.5 cA	1.0 cA	0.5 cA	0.6 cA	0.7 cA	0.7 cA	0.7 cA	30.3 bB	34.7 aB
Level of significance			Inoculants			Days of ensilage				Inoculants x Days of ensilage				SEM	
			<0.01			<0.01				<0.01				0.78	
Butyric acid (g/kg DM)															
12	0.8 aA	0.9 aA	1.2 aB	0.8 aB	1.5 aB	1.0 aB	1.2 aB	1.1 aB	1.2 aB	0.7 aC	1.3 aB	1.0 aA	2.4 aB	1.3 aB	1.1 aA
30	0.4 aA	0.9 aA	1.5 aB	2.1 aB	2.1 aB	1.5 aB	1.3 aB	1.9 aB	2.5 aB	1.8 aC	1.8 aB	0.4 aA	0.9 aB	1.3 aB	1.5 aA
61	0.0 bA	0.0 bA	3.3 aA	2.5 aB	4.9 aA	2.3 aB	2.6 aB	3.6 aB	3.6 aB	3.5 aB	3.2 aB	0.4 bA	1.9 bB	1.2 bB	1.3 bA
126	0.0 dA	0.0 dA	5.3 bA	7.0 bA	5.9 bA	5.1 bA	5.1 bA	6.3 bA	6.7 bA	9.9 aA	7.0 bA	0.9 dA	5.5 bA	5.4 bA	3.2 cA
Level of significance			Inoculants			Days of ensilage				Inoculants x Days of ensilage				SEM	
			<0.01			<0.01				<0.01				0.76	

a–d For each row, mean values with different lowercase letters are significant at P<0.05 by Scott–Knott test.
A–D For each column, mean values with different capital letters are significant at P<0.05 by Scott–Knott test.
[a] Control: without inoculant.
[b] Standard error of the means of two-way interactions (treatments with the strains and days of ensilage).
♦ *Lactobacillus plantarum.*
‡ *Lactobacillus brevis.*
* *Lactobacillus hilgardii.*

Table 7
Effects of inoculation with different strains and of different days of ensilage on the lactic acid bacteria and yeasts population in sugar cane silages.

Days of ensilage	Control[a]	UFLA SIL19 ♦	UFLA SIL32 ♦	UFLA SIL33 ‡	UFLA SIL34 ♦	UFLA SIL17 ‡	UFLA SIL24 ‡	UFLA SIL25 ‡	UFLA SIL27 ‡	UFLA SIL35 ♦	UFLA SIL41 ♦	UFLA SIL42 ♦	UFLA SIL46 ♦	UFLA SIL51 *	UFLA SIL52 *
Lactic acid bacteria (log CFU/g silage)															
12	8.55 aA	8.57 aA	8.53 aA	8.72 aA	8.12 aA	8.74 aA	8.77 aA	8.74 aA	8.85 aA	8.10 aA	8.19 aA	8.91 aA	8.34 aA	9.28 aA	9.24 aA
30	7.85 bA	6.19 cB	6.16 cB	6.31 cB	5.84 cB	6.02 cB	5.84 cB	6.14 cB	6.37 cB	5.69 cB	6.00 cB	7.43 bB	5.65 cB	9.04 aA	9.15 aA
61	8.16 aA	5.98 bB	6.44 bB	6.76 bB	7.09 bB	6.48 bB	5.55 bB	7.37 bB	7.02 bB	6.93 bA	6.82 bB	7.28 bB	6.43 bB	8.36 aA	8.40 aA
126	7.51 aA	7.33 aB	6.71 aA	5.33 bB	7.00 aB	6.75 aB	5.10 bB	7.39 bB	7.03 aB	6.05 aB	6.69 aB	7.02 aB	7.49 aA	4.30 bB	6.27 aB
Level of significance			Inoculants			Days of ensilage				Inoculants x Days of ensilage				SEM[b]	
			<0.01			<0.01				<0.01				0.47	
Yeasts (log CFU/g silage)															
12	5.88 aA	5.29 aA	6.07 aA	5.91 aA	6.08 aA	6.14 aA	5.42 aA	5.43 aA	5.25 aA	6.06 aA	6.22 aA	5.87 aA	6.13 aA	5.33 aA	5.46 aA
30	5.99 aA	6.31 aA	6.47 aA	5.88 aA	6.25 aA	6.00 aA	6.00 aA	5.57 aA	6.23 aA	6.41 aA	6.18 aA	6.71 aA	6.52 aA	5.17 aA	4.96 aA
61	5.38 bA	6.48 aA	6.16 aA	4.81 bB	4.98 bA	4.57 bB	5.35 bA	5.12 bA	4.58 bB	5.08 bA	5.20 bA	5.88 aA	6.22 aA	4.61 bA	4.83 bA
126	4.76 bA	5.61 aA	5.50 aA	4.39 bB	6.20 aA	4.68 bB	3.35 bB	5.49 aA	3.82 bB	6.13 aA	5.45 aA	5.96 aA	5.68 aA	4.13 bA	3.87 bA
Level of significance			Inoculants			Days of ensilage				Inoculants x Days of ensilage				SEM	
			<0.01			<0.01				0.23				0.44	

a–d For each row, mean values with different lowercase letters are significant at P<0.05 by Scott–Knott test.
A–C For each column, mean values with different capital letters are significant at P<0.05 by Scott–Knott test.
[a] Control: without inoculants.
[b] Standard error of the means of two-way interactions (inoculants and days of ensilage).
♦ *Lactobacillus plantarum.*
‡ *Lactobacillus brevis.*
* *Lactobacillus hilgardii.*

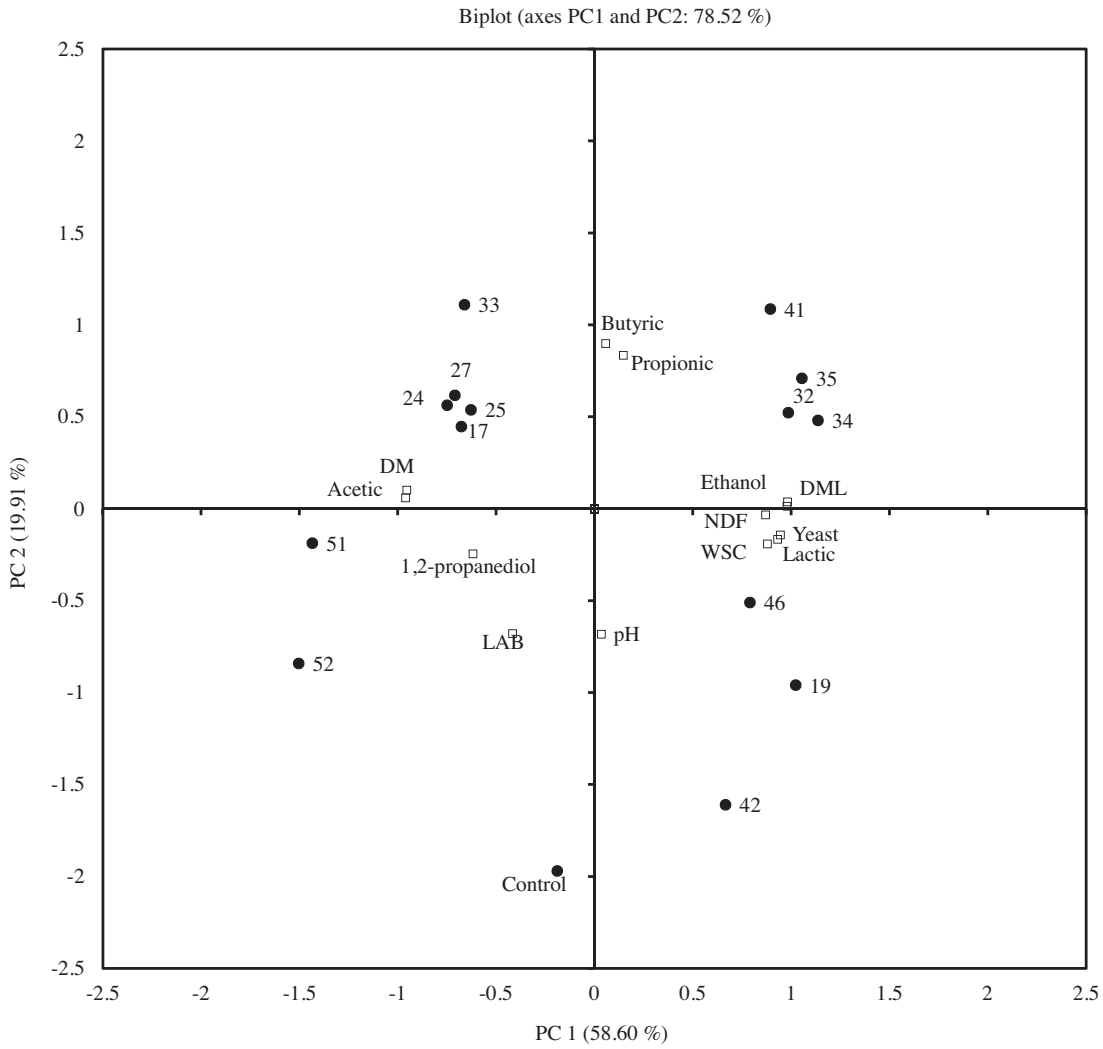

Fig. 1. Principal component analysis (PCA) of the fermentation products, dry matter (DM), DM loss (DML), neutral detergent fiber (NDF), water soluble carbohydrates (WSC), lactic acid bacteria (LAB) and yeasts population in sugar cane silage treated with wild strains of lactic acid bacteria.

et al., 2010) and other forages (Saarisalo et al., 2007; Li and Nishino, 2011a). Two strains (UFLA SIL51 and UFLA SIL52) were identified as *L. hilgardii*. This species was for the first time selected as an inoculant for sugar cane silage. Some studies have demonstrated the occurrence of *L. hilgardii* in lactic fermentations under acidic and anoxic conditions closely resembling those that prevail in silage where several other species of *Lactobacillus* are also present (Leroi and Pidoux, 1993; Baruzzi et al., 2000; Mtshali et al., 2010). Heinl et al. (2012) verified that the metabolism of both *L. hilgardii* and *L. buchneri* are similar and possess the ability to degrade lactic acid and to produce acetic acid and 1,2-propanediol in anaerobic conditions.

The initial grouping of strains based on the type of acid produced did not relate to further strain identification or to the carbohydrate fermentation standards. As shown in Tables 1 and 2, strains of the same species may have different fermentation standards and produce different types of acid as main products of metabolism. These inconsistencies can be attributed to the fact that physiological properties are based on phenotypic expression that is possibly influenced by regulation, whereas the PCR methods are strictly based on DNA sequences (Mtshali et al., 2010). Therefore, bacteria of the same species can produce a range of different enzymes that influence the organisms' abilities to use different substrates. This also explained the divergence between the biochemical and 16S rDNA sequencing identifications of two evaluated strains. The UFLA SIL51 and UFLA SIL52 strains were biochemically identified as *L. buchneri*; however, the results of the sequencing indicated they were strains of the species *L. hilgardii*. That these strains are physiologically and genetically correlated (Sohier et al., 1999; Heinl et al., 2012) might explain the ambiguity in the identification. As observed by Ávila et al. (2010), the fermentative variety of the studied strains confirmed the need to select for strain, not species, when establishing a starter culture.

The DM, WSC, and NDF-NDF content in sugar cane found in this study prior to ensilage was low but within the range of observations reported previously in the literature (Ávila et al., 2009; Carvalho et al., 2012). These characteristics are

appropriate to provide an adequate fermentation, mainly due to the high concentration of soluble carbohydrates. In the present study, the LAB count found in the fresh sugar cane (7.51 log CFU/g forage) was above that observed previously (Carvalho et al., 2012). The LAB naturally present in forage crops influences silage quality and is responsible for silage fermentation. The yeasts and filamentous fungi populations found were within the variation described in the literature for sugar cane (Ávila et al., 2009).

DM loss during the fermentation of sugar cane constitutes the main problem in the ensilage of this forage (Kung and Stanley, 1982; Pedroso et al., 2005). Thus, this should be the main characteristic evaluated in the selection of inoculant strains for the culture. The *L. brevis* (UFLA SIL17 and UFLA SIL24) and *L. hilgardii* (UFLA SIL51 and UFLA SIL52) strains showed the best results (*i.e.*, lower DM losses, higher DM concentrations, and lower NDF-NDF concentrations). These strains presented obligatory heterofermentative metabolism, wherein they were able to produce gas during the growth in MRS broth. Heterofermentative LAB produces ethanol and acetic acid in addition to lactic acid (Axelsson, 1998). Weak organic acids, such as acetic acid, act to inhibit the growth of yeasts (Danner et al., 2003), which are responsible for the DM losses in sugar cane silages.

Increase in DM losses and NDF–NDF, and decrease in DM concentrations throughout the sugar cane fermentation process are common during ensilage (Pedroso et al., 2005). In the present study, the increases in the DM losses and in the NDF-NDF concentrations, and the decreases in the DM concentrations were more intense until day 61 of ensilage. At this point, a stabilization was observed, as evaluated and confirmed at 126 days of ensilage.

The decrease in the concentration of carbohydrates during the ensilage in all silages occurred quickly. By day 12 of fermentation, 50% of the WSC were consumed, and by day 126, the consumption had reached 94%. Pedroso et al. (2005) also observed a decrease in WSC concentration in the beginning of the fermentation of this forage. In the silages inoculated with *L. hilgardii* strains (UFLA SIL51 and UFLA SIL52), the utilization of carbohydrates occurred more swiftly than in the control and silages inoculated with the remaining LAB strains. Consequently, at 12 days of fermentation, a lower concentration of carbohydrates and a higher LAB population were recorded in the silages treated with these strains.

An association was observed between the lower populations of yeasts and lower concentrations of ethanol present in the silages inoculated with the UFLA SIL51 and UFLA SIL52 strains. During the ensilage, the concentration of ethanol increased in all silages, with the exception of those inoculated with UFLA SIL51 and UFLA SIL52 strains. The presence of ethanol in the silages is undesirable; despite this compound being a source of energy for ruminants (Kristensen et al., 2007), it also indicates the growth of yeasts and DM losses. Although the heterofermentative LAB produce ethanol, yeasts are mainly associated with the presence of this metabolite in sugar cane silages (Ávila et al., 2009; Pedroso et al., 2005). The higher concentration of ethanol observed in the silages inoculated with *L. plantarum* strains possibly resulted from the fermentative activity of the yeasts. Silages inoculated with *L. plantarum* showed lower concentrations of acetic acid compared to those inoculated with obligatory heterofermentative strains. This may have favored yeast development since high acetic acid concentrations are effective in inhibiting their growth (Moon, 1983).

The presence of lactic acid in the silage is considered an indicator of fermentation but not necessarily successful fermentation. For a successful fermentation, values above 30 g of lactic acid/kg DM are typically desired for low DM content silages (Cherney and Cherney, 2003). In the silages treated with the *L. plantarum*, *L. brevis* (UFLA SIL33), and *L. hilgardii* (UFLA SIL51 and UFLA SIL52) strains, this concentration was achieved by day 12 of fermentation, while the control silage resulted in only 17.2 g/kg DM by that day. At day 126 of ensilage, the highest concentrations of lactic acid were observed in the silage inoculated with *L. plantarum* (UFLA SIL19, UFLA SIL32, UFLA SIL34, UFLA SIL35, UFLA SIL41, UFLA SIL42, and UFLA SIL46). The species *L. plantarum* has a facultatively heterofermentative metabolism. It is able to ferment hexoses and pentoses, as it constitutively expresses aldolase and phosphoketolasei enzymes (Axelsson, 1998). Lactic acid is the main product formed when the glycolytic pathway is used by LAB to obtain energy from hexoses (Axelsson, 1998). The *L. brevis* and *L. hilgardii* strains have an obligatory heterofermentative metabolism, that is, they make use of the pentose phosphate pathway and produce proportionately smaller quantities of lactic acid than *L. plantarum*.

Weak organic acids such as propionic, acetic, and butyric acids inhibit the growth of yeasts and filamentous fungi (Moon, 1983). However, the presence of butyric acid in silage is undesirable because it reduces the acceptability of the feed and decreases the intake (Weiss et al., 2003). The increase in the butyric acid concentration as a result of the inoculation with LAB strains has been observed by Carvalho et al. (2012). Concentrations of butyric acid lower than 25 g/kg DM in silages are tolerable (Weiss et al., 2003). Thus, the concentrations of butyric acid observed in the present study could be considered low.

The silages treated with the obligatory heterofermentative strains showed the highest concentrations of acetic acid. In particular, those silages treated with the *L. hilgardii* UFLA SIL51 and UFLA SIL52 strains resulted in 55% more acetic acid than the control and those treated with the remaining strains. Some obligatory heterofermentative LAB and other facultatively heterofermentative strains do not express (or express in a constitutive way) acetaldehyde dehydrogenase, which is one of the enzymes responsible for the reduction of acetyl-CoA into ethanol. Thus, the production of ethanol was virtually null in this group of LAB (Axelsson, 1998; Heinl et al., 2012). Consequently, there was an increase in the concentration of acetic acid as a final fermentation product.

The increase in acetic acid concentration throughout the fermentation process could have resulted from the anaerobic conversion of the lactic acid into acetic acid and 1,2-propanediol generally related to *L. buchneri* (Oude Elferink et al., 2001; Heinl et al., 2012). The LAB degradation of the lactic acid might be associated with the preservation of cell viability (Oude

Elferink et al., 2001). This might explain why the concentrations of lactic acid were lower and the concentrations of 1,2-propanediol and acetic acid higher in the silages inoculated with UFLA SIL51 and UFLA SIL52 than in the control.

The formation of the 1,2-propanediol is initiated by the conversion of lactate into lactaldehyde by a lactaldehyde dehydrogenase (Heinl et al., 2012). The second step of 1,2-propanediol formation is the conversion of lactaldehyde into 1,2-propanediol, which is catalyzed by a putative lactaldehyde reductase (Oude Elferink et al., 2001). This metabolic pathway has been described for the *L. buchneri* and *L. hilgardii* species, suggesting that the inoculated microorganisms must act in an efficient manner during the fermentation process.

The concentration of propionic acid in the different silages did not show large variation, even in the silages treated with the *L. plantarum*, *L. brevis*, and *L. hilgardii* strains. In the case of silages not inoculated with *Propionibacterium*, the presence of propionic acid might also be associated with the presence of *Lactobacillus diolivorans* (Krooneman et al., 2002), which is able to use 1,2-propanediol to produce propionic acid.

The silages inoculated with the UFLA SIL51 and UFLA SIL52 strains resulted in higher LAB populations until day 61 of fermentation. The WSC concentrations in the silages treated with these strains also decreased more rapidly in the beginning of the fermentation process, which coincided with the high LAB population observed in the same period. As these strains were able to ferment the D-saccharose, it is likely that they used this carbohydrate to sustain their growth without requiring previous metabolic processing by other microorganisms. The filamentous fungi populations were lower than the minimum detectable counting in all silages ($\leq$2 log CFU/g). This is in agreement with the results reported by Ávila et al. (2010). The inhibition of fungal growth can occur due to low oxygen levels and lactic and acetic acid concentrations or through the actions of LAB-produced bacteriocins present in the silages (Strom et al., 2002).

The correlation between high ethanol content and NDF-NDF concentrations with the highest DM loss in sugar cane silage has been observed in other studies (Pedroso et al., 2005). This study was the first to observe that in sugar cane silage, lower DM loss was correlated with the highest concentrations of 1,2-propanediol and acetic acid. This is unlike in the present study where, in corn (Li and Nishino, 2011b) and grass (Driehuis et al., 2001), silages with high concentration of 1,2-propanediol and acetic acid were associated with high DM loss.

5. Conclusion

The different behaviors of the LAB strains during carbohydrate fermentation and throughout the ensilage period reinforce the importance of choosing specific strains for each forage plant. The use of facultatively heterofermentative LAB, particularly the species *L. plantarum*, was not beneficial for the sugar cane ensilage. In these treatments, the highest DM losses, ethanol concentrations, and yeast populations were observed. The presences of ethanol, lactic acid, and yeast populations were most associated with higher DM loss among all evaluated variables. Silage inoculation with obligatory heterofermentative strains produced the best results. In particular, the *L. hilgardii* UFLA SIL51 and UFLA SIL52 strains showed superior results, reducing 29% the DM losses compared to the uninoculated silage. These strains are promising for use as microbial inoculants in sugar cane silages.

Conflict of interest

The authors have no conflicts of interest to declare.

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