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Effect of Various Strains of *Lactobacillus buchneri* on the Fermentation Quality and Aerobic Stability of Corn Silage

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Abstract: The aerobic deterioration of silage nutrients is inevitable in tropical countries, causing negative consequences in animal production systems. Aiming to minimize the losses, the effects of *Lactobacillus buchneri* strains on fermentation profile and aerobic stability of corn silages were evaluated. The experiment was conducted under a completely randomized design with 13 treatments and three replicates. The treatments were noninoculated, commercial *L. buchneri* (CI), and 11 wild strains of *L. buchneri*: LB-56.1, LB-56.2, LB-56.4, LB-56.7, LB-56.8, LB-56.9, LB-56.21, LB-56.22, LB-56.25, LB-56.26, and LB-56.27. The treatments could be divided into three different groups according to silage pH and acetic acid concentration. Silages inoculated with LB-56.1, LB-56.4, and LB-56.9 presented higher pH, whereas intermediate values were observed for LB-56.2, LB-56.7, and LB-56.8. The highest acetic acid production was observed for LB-56.1 and LB-56.7. On the other hand, lowest concentrations were found for CI, LB-56.22, LB-56.25, LB-56.26, and LB-56.27. Higher amounts of NH_3–N were observed for LB-56.8, LB-56.21, LB-56.22, and LB-56.27 silages than others. Silage inoculation with CI, LB-56.1, LB-56.2, LB-56.4, LB-56.8, LB-56.9, and LB-56.25 strains had higher aerobic stability than others (59.7 vs. 41.2 h). The *L. buchneri* strains LB-56.1, LB-56.2, LB-56.4, LB-56.8, LB-56.9, and LB-56.25 provided potential features to improve the aerobic stability of corn silage.

Keywords: dry matter recovery; microbial inoculant; pH; *Zea mays*

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1. Introduction

Corn silage has become the predominant forage used in the production of ruminant's diets worldwide because of minimized risks of production, elevated yield per area, and flexibility of harvesting corn for forage or grain [1]. Moreover, uniquely, in comparison with other forages, corn silage offers dairy nutritionists the opportunity to provide high energy along with physically effective neutral detergent fiber (NDF) [2].

Due to the high temperature and relative humidity in tropical countries, the aerobic deterioration of silage nutrients is inevitable, causing significant losses and negative consequences in animal production systems [3].

It is generally accepted that yeasts are responsible for the onset of aerobic deterioration in silages [4]. After ensiling, the yeasts compete with other microorganisms for fermentable substrates, and during the initial weeks of fermentation, the population can reach up to 10^7 colony-forming units (cfu)/g of fresh weight, with a gradual decrease during the subsequent storage stages [5]. The survival of these microorganisms during the fermentation

process depends on the degree of anaerobiosis, the pH, and the types and concentrations of organic acids.

Under aerobic conditions, spoilage yeasts are able to oxidize sugars and lactic acid, resulting in the production of CO_2, H_2O, and heat, and thus causing spoilage and loss of important nutritional components [6]. The concern with aerobic stability is not limited to dry matter (DM) losses, because the development of microorganisms, such as bacteria belonging to the genera *Bacillus, Clostridium,* and *Listeria,* and some filamentous fungi may influence the hygienic quality of silage by toxins production [7].

Thereby, in the mid-1990s, Muck [8] suggested that the addition of *Lactobacillus buchneri* to silages might prove valuable in improving aerobic stability and it has become the dominant species used in obligate heterofermentative lactic acid bacteria (LAB) inoculants [9].

Although there are a range of inoculants based on *L. buchneri* available on the market, recent studies testing different strains as silage inoculants on a variety of forages in tropical regions are still inconsistent [10–12]. Indeed, in a recent meta-analysis, Arriola et al. [13] concluded that silage inoculation with *L. buchneri* increased aerobic stability of all silages except tropical grasses. Thus, more research is needed on the use of new microorganisms, especially those isolated under tropical conditions with antimicrobial activity that may result in significant effects on fermentation parameters and aerobic deterioration, and, consequently, improvements in silage quality.

In a previous study conducted in our lab, Silva et al. [14] isolated 15 strains of *L. buchneri* from corn silage: four of them were evaluated as microbial inoculants in corn silage and four in sugarcane silage. The strains LB-56.1 and LB-56.4 were promising microbial inoculants in sugarcane fermentation, but the effects of these microorganisms were not evaluated in corn silage fermentation. In this context, aiming to test the viable *L. buchneri* strains resulting from this previous study, we evaluated the effects of eleven *L. buchneri* strains on microbial populations, fermentation profile, chemical composition, DM recovery, and aerobic stability of corn silages after 90 days of fermentation.

2. Materials and Methods

The experiment was conducted between March and June 2016 at the Department of Animal Science of the Federal University of Vicosa (Vicosa, MG, Brazil), located at 20°45′ south latitude, 42°51′ west longitude and 648 m above sea level. The annual precipitation and average temperature in the year of the experiment were 1235.4 mm and 20.7 °C, respectively.

The experiment was carried out under a completely randomized design with three replicates and 13 treatments. The evaluated treatments were the following: noninoculated (CRTL), commercial *L. buchneri* strain (CI), and 11 wild strains of *L. buchneri*: LB-56.1, LB-56.2, LB-56.4, LB-56.7, LB-56.8, LB-56.9, LB-56.21, LB-56.22, LB-56.25, LB-56.26, and LB-56.27. The wild strains of *L. buchneri* were isolated from corn silage belonging to the culture collection of the Forage Laboratory of the Federal University of Vicosa. The sequences of the strains are deposited in the GenBank database, and their phenotypic characteristics are described in the study conducted by Silva et al. [14]. The commercial inoculant Lalsil AS (Lallemand Animal Nutrition, Inc., Blagnac, France), which contains *L. buchneri* CNCM-I 4323 1.0×10^{11} cfu/g and sucrose, was used based on manufacturer recommended dosage to compare its effectiveness with new strains.

The wild strains were cultured in Man Rogosa and Sharp (MRS) broth for 14 h, and then the inoculum was standardized using a spectrophotometer (630 nm) at an optical density of 0.05, into 20 mL of MRS broth and cultured for additional 14 h. With this, the amount of inoculum needed to reach the theoretical application rate of 10^5 cfu/g of fresh forage, the same rate of the commercial inoculant, was centrifuged at $1000\times$ *g* for 10 min and the supernatant was discarded.

Corn plants were harvested from the dairy unit of the Federal University of Vicosa with an average DM of 273 g/kg on fresh matter basis, using a one-row pull-type forage

harvester (JF-92 Z10; Staalmeester, Hartbeesfontein, South Africa) with a theoretical length chop of approximately 15 mm. Randomly, the chopped corn was divided in 39 piles of 10 kg and three replicated piles (each pile treated individually) were prepared for each treatment. The study complies with the Brazilian regulations for plant research. All methods were carried out in accordance with relevant guidelines and regulations.

Inoculants were diluted in 50 mL of water and applied with the aid of a hand sprayer. The same water quantity was applied to the CTRL silages. Eight kilograms of the treated forage were ensiled in plastic buckets (experimental mini-silos) with 10 L-capacity aiming at a density of 218 kg of DM/m^3 and sealed with plastic lids. The chopped forage was compacted in the buckets by trampling and was stored in a temperature range of 23–27 °C for 90 days.

Corn samples before ensiling and the silages were dried in a forced-air oven at 55 °C for 72 h and milled in a Wiley mill (Thomas-Wiley Mill Co., Philadelphia, PA, USA) with a sieve of 1 mm. The DM (method 934.01), crude protein (CP; method 984.13), and acid detergent fiber (ADF; method 973.18) were determined as described by AOAC [15]. The neutral detergent fiber (NDF) content was determined using heat-stable α-amylase without use of sodium sulfite and was corrected for residual ash [16]. Correction of the NDF and ADF for nitrogen compounds were performed according to Licitra et al. [17].

Twenty-five grams of the fresh forage and silage samples from each mini-silo were blended with 225 mL of sterile Ringers solution (Oxoid, Basingstoke, UK) for 1 min. The aqueous extract was divided in two portions; one of them was used to measure the pH using a potentiometer, and the concentrations of ammonia nitrogen (NH_3–N; [18]) and water-soluble carbohydrates (WSC; [19]) were determined.

For the organic acids, ethanol, and 1,2-propanediol quantifications, the aqueous extracts of the silages were centrifuged at 12,000 g for 10 min [20]. The analysis was performed in a high-performance liquid chromatography (Shimadzu Scientific Instruments, Columbia, MD, USA) equipped with SPD-10A VP, UV–Vis detector (Shimadzu Corporation, Analytical Instruments Division, Kyoto, Japan), using a wavelength of 210 nm, with a 30 cm × 4.5 mm diameter HPX-87H column (Bio-Rad Laboratories, Hercules, CA, USA) at a pressure of 63 kgf. The mobile phase used was sulfuric acid (H_2SO_4) 0.05 mM with a flow of 0.8 mL/min and an injected volume of 20 µL.

The second portion of the fresh aqueous extracts was used to quantify the LAB, enterobacteria, yeast, and mold populations. Serial dilutions were made in Ringers solution and the plating by the pour plate technique was carried out in different culture media.

Cultivation of the LAB population was performed in *Lactobacilli* MRS agar (Difco, Detroit, MI, USA) at 37 °C for 48 h; culture of enterobacteria was performed in Violet Red Bile agar (Difco, Detroit, MI, USA) at 37 °C for 24 h. The Dextrose Potato Agar (Difco, Detroit, MI, USA) media containing 1.5% of tartaric acid solution (10% *w*/*v*) was used to determine the yeast and mold counts after 72 and 120 h, respectively, at 25 °C. The number of cfu was determined on plates containing between 25 and 250 colonies.

The DM recovery was calculated according to Jobim et al. [21]. After 90 days of fermentation, two kg of silage from each silo were collected and placed in plastic buckets without a lid; the buckets were covered with two layers of cheesecloth and kept in a room at 21 ± 1 °C for 7 days. Temperatures were measured every 15 min using data loggers (Impac, model MI-IN-D-2-L; São Paulo, Brazil), inserted into the silages mass at geometric center.

The aerobic stability was denoted by the time (h) before a 2 °C rise in silage temperature above ambient temperature (21 °C). The maximum temperature (Tmax) reached by the mass and the time it took to reach those temperatures (HTmax) were also measured. After 7 days of air exposure, 25 g of representative samples from each bucket were homogenized in 225 mL of sterile Ringers solution in industrial blender for 1 min, and the pH and organic acids were measured as described previously. Yeast and mold counts were presented together (YM) due to the impossibility of distinguishing populations in these samples.

The data were analyzed using the GLM procedure of SAS® (v. 9.4 SAS Institute Inc., Cary, NC, USA). The general model was given by: Yij = µ + Ii + eij, where Yij = response

variable; μ = general mean; Ii = effect of inoculant i; and eij = random error. To obtain results without ambiguity among treatments, the estimated means were grouped through Scott–Knott method implemented in the R software [22].

3. Results and Discussion

The initial pH of corn forage averaged 5.83 and the DM, CP, NDF, and ADF contents averaged 273 g/kg, 54.5, 550, and 314 g/kg of DM, respectively (data not shown). The WSC concentration (92.4 g/kg of DM) before ensiling was sufficient to ensure adequate ensiling [23].

The suitable population of microorganisms in the silage dictates the sense of the fermentation and the proportions of organic acids that will be produced, influencing the deterioration and the consumption of silages by the animals. The initial LAB population (7.4 log cfu/g fresh weight) was higher than the minimum established by Muck [8] (5.0 log cfu/g fresh weight) as adequate for the occurrence of good fermentation of silage. Moreover, the initial populations of enterobacteria, yeast, and mold averaged 7.25, 6.44, and 5.70 log cfu/g fresh weight, respectively (data not shown).

The chemical composition and DM recovery of silages after 90 days of fermentation are shown in Table 1. The DM, CP, NDF, ADF, and DM recovery were not affected ($p > 0.05$) by the treatments and averaged 268 g/kg, 55.4, 505, and 329 g/kg of DM and 97.0%, respectively.

Table 1. Chemical composition (g/kg of dry matter, unless otherwise stated) and dry matter recovery of corn silage treated with *Lactobacillus buchneri* strains after 90 days of fermentation.

Treatment	Items				
	DM †, g/kg	CP ‡	NDF §	ADF ¶	DM rec ¥, %
CTRL	257	54.7	549	329	91.9
CI	260	58.4	505	333	95.9
LB-56.1	260	56.9	515	354	92.7
LB-56.2	268	56.7	520	332	95.5
LB-56.4	262	57.5	496	330	95.0
LB-56.7	267	55.1	505	297	88.7
LB-56.8	268	54.7	511	319	97.1
LB-56.9	265	56.7	488	341	97.0
LB-56.21	269	51.5	510	349	98.8
LB-56.22	272	53.9	497	295	99.7
LB-56.25	270	56.9	486	280	97.8
LB-56.26	271	55.4	497	283	99.1
LB-56.27	276	55.1	491	282	97.6
SEM	2.339	0.551	5.361	5.952	4.318
ANOVA *p*-value	0.97	0.71	0.79	0.08	0.20

† Dry matter; ‡ Crude protein; § Neutral detergent fiber; ¶ Acid detergent fiber; ¥ Dry matter recovery; CTRL: noninoculated; CI: commercial inoculant—Lalsil AS; LB-56.1-56.27—wild *Lactobacillus buchneri* strains.

Treatment effects ($p < 0.05$) were observed for pH, WSC, NH_3–N, lactic, acetic and propionic acids, ethanol, and 1,2-propanediol concentrations. The fermentation patterns of silages after 90 days of storage are shown in Table 2. Although approaching practical results, the concentrations of lactic acid, ethanol, and WSC were not significant under a Scott–Knott framework.

Table 2. Fermentation patterns (g/kg of dry matter, unless otherwise stated) of corn silage treated with *Lactobacillus buchneri* strains after 90 days of fermentation (SEM, standard error of mean).

	Items								
	pH	**WSC** [†]	**NH_3–N** [‡]	**LA** [§]	**AA** [¶]	**BA** [¥]	**PA** [€]	**ET** [ϰ]	**1,2-PD** [£]
CTRL	3.65 [c]	10.8	80.4 [b]	59.1	12.4 [b]	0.27	0.80 [e]	17.2	3.06 [a]
CI	3.70 [c]	6.90	86.3 [b]	29.0	10.9 [c]	0.23	7.55 [d]	11.7	2.56 [b]
LB-56.1	3.84 [a]	8.10	69.9 [b]	37.8	25.3 [a]	0.23	30.2 [a]	26.2	2.75 [b]
LB-56.2	3.80 [b]	5.40	74.9 [b]	34.6	14.7 [b]	0.23	15.0 [c]	14.9	2.46 [b]
LB-56.4	3.85 [a]	7.90	80.1 [b]	31.8	14.0 [b]	0.27	16.5 [c]	16.2	2.40 [b]
LB-56.7	3.76 [b]	7.00	72.3 [b]	43.0	20.8 [a]	0.23	15.0 [c]	7.10	2.36 [b]
LB-56.8	3.81 [b]	7.10	100 [a]	39.7	15.4 [b]	0.27	21.1 [b]	16.5	2.30 [b]
LB-56.9	3.83 [a]	8.20	79.4 [b]	36.3	14.4 [b]	0.25	16.2 [c]	13.6	2.46 [b]
LB-56.21	3.71 [c]	7.80	99.7 [a]	53.4	13.3 [b]	0.23	3.50 [e]	15.7	2.40 [b]
LB-56.22	3.70 [c]	8.30	95.2 [a]	38.1	8.30 [c]	0.25	0.80 [e]	13.4	2.60 [b]
LB-56.25	3.71 [c]	7.70	81.4 [b]	41.1	6.60 [c]	0.23	1.25 [e]	8.00	3.35 [a]
LB-56.26	3.67 [c]	8.80	77.3 [b]	35.0	8.20 [c]	0.23	0.70 [e]	6.05	2.43 [b]
LB-56.27	3.68 [c]	8.70	90.7 [a]	50.6	11.1 [c]	0.27	1.25 [e]	14.5	2.26 [b]
SEM	0.016	0.254	2.762	3.912	1.353	0.010	1.655	1.649	0.156
ANOVA *p*-value	<0.01	0.009	0.01	0.04	<0.01	0.99	<0.01	<0.01	0.0003

[a–e] Means within treatments followed by the same letters group together according to the Scott–Knott clustering procedure ($p < 0.05$); [†] Water-soluble carbohydrate; [‡] Ammonia nitrogen (g/kg of total nitrogen); [§] Lactic acid; [¶] Acetic acid; [¥] Butyric acid; [€] Propionic acid, [ϰ] Ethanol, [£] 1,2-propanediol; CTRL: noninoculated; CI: commercial inoculant—Lalsil AS; LB-56.1-56.27—wild *Lactobacillus buchneri* strains.

The lactic acid observed in silages was lower than 60–100 g/kg of DM and the ethanol concentrations were within the range of 10–30 g/kg of DM suggested by Kung et al. [24] for corn silage. The ethanol ingested by the animals can be converted to acetic acid in the rumen or absorbed by the rumen wall to subsequently be converted to milk fat or used for body metabolism or growth [25].

According to the results provided by Scott–Knott clustering procedure, treatment groupings were observed for pH and acetic acid concentration. Silages inoculated with LB-56.1, LB-56.4, and LB-56.9 had the highest pH, whereas intermediate values were observed in LB-56.2, LB-56.7, and LB-56.8 silages. However, all pH values were within the range of 3.6 and 4.0, which reflects adequate fermentation for restricting the growth of undesirable microorganisms [24].

Acetic acid is the acid found in the second-highest concentration in silage, usually ranging from 10 to 30 g/kg of DM [24]. The highest acetic acid production was observed in silages inoculated with LB-56.1 and LB-56.7, whereas the lowest concentrations were found in CI, LB-56.22, LB-56.25, LB-56.26, and LB-56.27 silages. Butyric acid was not affected ($p = 0.99$) by treatment, and averaged 0.23 g/kg of DM.

Propionic acid was grouped into five groups with unexpectedly high concentrations in some inoculated silages. Silages inoculated with the strain LB-56.1 had the highest propionic acid concentration, followed by LB-56.8 in the second group, LB-56.2, LB-56.4, LB-56.7, and LB-56.9 in the third group, CI in the fourth, and the remaining treatments presented the lowest production.

High concentration of propionic acid (>3–5 g/kg of DM) is most commonly found in clostridial fermentation. Besides the presence of butyric acid, clostridial silages are often characterized by lower concentrations of lactic acid and higher pH and acetic acid, NH_3–N, and soluble protein concentrations [24], characteristics that were not observed in our silages.

The same authors stated that high concentrations of propionic acid may be observed in silages due to the conversion of glucose and lactic acid to propionic and acetic acids by

some Propionibacteria or also due to metabolization of 1,2-propanediol producing propanol and propionic acid by *Lactobacillus diolivorans* [26].

The propionic acid is the best single silage DM intake predictor [27]. Although the mechanism of how this acid affects the intake remains unsolved, the authors suggested that it must be associated with adverse effect caused by secondary fermentation of silage rather than being a straightforward effect of the acid. Evidence of secondary fermentation was not observed in our study based on the evaluated parameters.

A variety of microorganisms can produce 1,2-propanediol, including some species of clostridia and yeasts [28,29]. However, in typical silages, 1,2-propanediol is most likely the result of metabolism of lactic acid by *L. buchneri*. The higher amount of 1,2-propanediol was unexpected in CTRL silage, but the naturally occurring populations of *L. buchneri* can sometimes result in low concentration of this alcohol (1–3 g/kg of DM). In addition, the natural occurrence of *L. diolivorans* in silages can lead to underestimation of this alcohol [24], as described above.

During the fermentation process, plant enzymes primarily mediate the initial proteolysis, while subsequent amino acid degradation occurs by the action of microorganisms [30]. The NH_3–N formed in this process alters the fermentation course, inhibiting a rapid drop in the pH of the ensiled mass, affects silage consumption by animals, and reduces the efficiency of the use of nitrogen for protein synthesis by ruminal microorganisms [31]. Greater amounts of NH_3–N were observed in silages inoculated with LB-56.8, LB-56.21, LB-56.22, and LB-56.27 strains than others (96.4 vs. 78.0 g/kg of total nitrogen).

Microbial fermentation in the silo produces an array of end products and can change many nutritive aspects of a forage. The microbial population counts are present in Figure 1. Population of LAB differed ($p = 0.003$) among treatments. Lower LAB counts were observed in CTRL, LB-56.4, LB-56.22, LB-56.26, and LB-56.27 silages than others. The populations of enterobacteria, yeast, and mold averaged 0.43, 5.40, and 1.02 log cfu/g of fresh weight, respectively.

The growth of enterobacteria in silages is undesirable because they compete with the LAB for the available sugars and degrade protein [32]. In our study, the low counts of this microorganism were expected due to the rapid and sufficient drop in silage pH during the ensiling process [33].

Enumeration of yeasts and molds may be useful for silage quality evaluation because high counts of yeasts in silages are usually associated with high concentrations of ethanol, and their numbers are often inversely related to the aerobic stability of the ensiled material.

Two groups of treatments were observed for aerobic stability in our study (Figure 2). Silages inoculated with CI, LB-56.1, LB-56.2, LB-56.4, LB-56.8, LB-56.9, and LB-56.25 were more aerobically stable compared with noninoculated silage and other strains (59.7 vs. 41.2 h, Figure 2). The inoculation of corn with the strain LB-56.1 resulted in silages 14 h more stable than the commercial strain. Although this difference was not significant under the Scott–Knott framework, this improvement cannot be discarded in practice.

The differences in aerobic stability were not accompanied by changes in yeast and mold counts in our study. Silva et al. [14] also observed the absence of inoculant effect on these microorganisms after fermentation process due to the high initial population in raw material. The numbers of yeasts and molds, for the current study, are within the normal range usually reported for these silages [6].

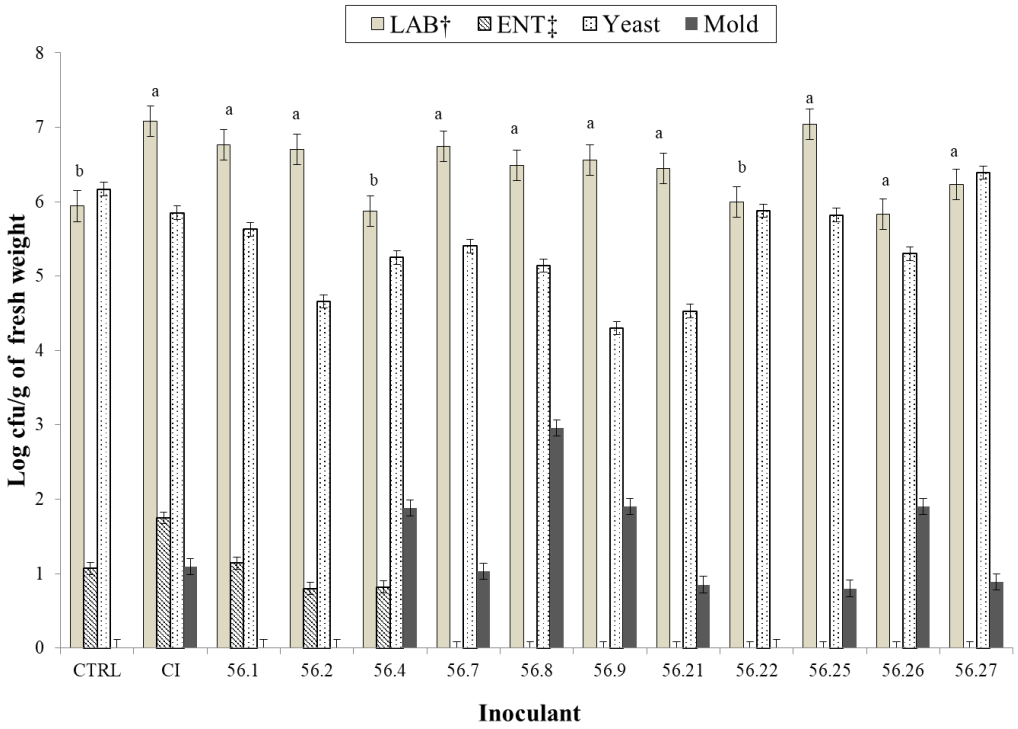

Figure 1. Microbial populations of corn silage treated with *Lactobacillus buchneri* strains after 90 days of fermentation. [a,b] Means within columns followed by the same letter group together according to the Scott–Knott clustering procedure ($p < 0.05$); cfu: colony-forming unit; † lactic acid bacteria—ANOVA p-value = 0.003, SEM ± 0.41; ‡ enterobacteria—ANOVA p-value = 0.43, SEM ± 0.16; yeast—ANOVA p-value = 0.48, SEM ± 0.18; mold—ANOVA p-value = 0.16, SEM ± 0.22; CTRL: noninoculated; CI: commercial inoculant—Lalsil AS; LB-56.1-56.27—wild *Lactobacillus buchneri* strains.

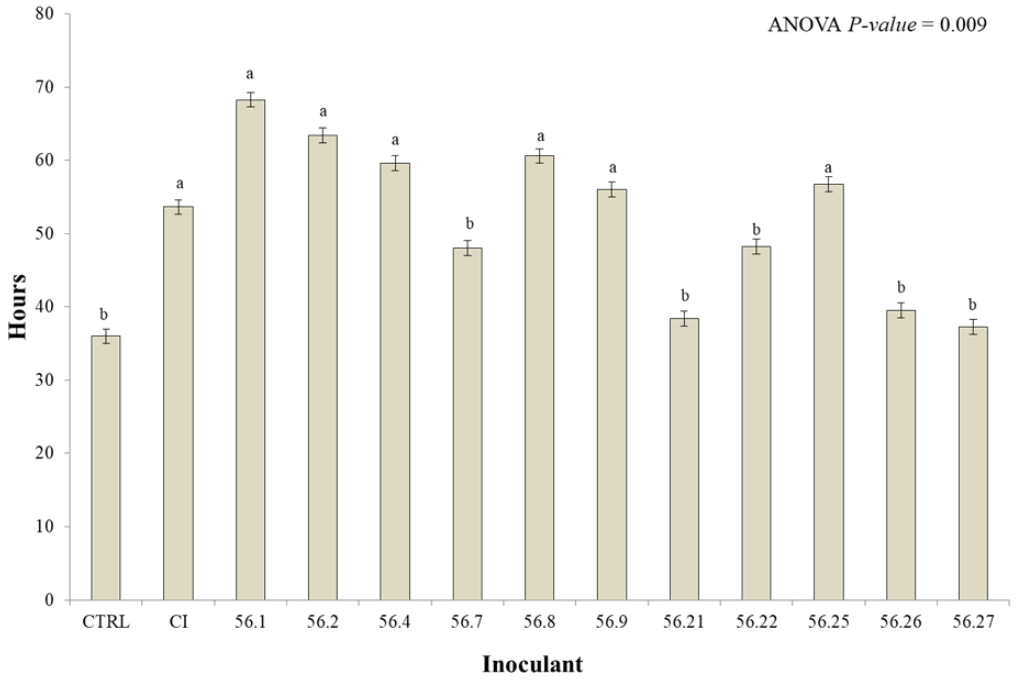

Figure 2. Aerobic stability of corn silage treated with *Lactobacillus buchneri* strains after 7 days of air exposure at 21 °C (SEM ± 3.803). [a,b] Means within columns followed by the same letter group together according to the Scott–Knott clustering procedure ($p < 0.05$); CTRL: noninoculated; CI: commercial inoculant—Lalsil AS; LB-56.1-56.27—wild *Lactobacillus buchneri* strains.

Indeed, aerobic stability has not always been well correlated with numbers of yeasts in some studies [34]. According to the same authors, the enumeration of yeast population in silages needs to be interpreted carefully because analytical laboratories enumerate the total number of yeasts but do not differentiate lactate and nonlactate assimilator microorganisms. In addition, the growth of yeast on selective agar does not necessarily reflect their metabolic capabilities in silage. Lastly, other microorganisms, such as *Acetobacter* bacteria, are able to oxidize lactate and acetate to carbon dioxide and water, thus initiating the aerobic spoilage.

The increased aerobic stability observed in silages inoculated with CI, LB-56.1, LB-56.2, LB-56.4, LB-56.9, LB-56.21, and LB-56.26 strains may be related to inhibiting effect on specific yeast species and other deteriorating microorganisms due to the accumulation of known compounds, such as acetic and propionic acids, and other antimicrobial metabolites, such as 3-hydroxydecanoic acid, 3-(R)-hydroxytetradecanoic acid, 4-hydroxybenzoic acid, vanillic acid, 2,3-butanedione, acetaldehyde, and bacteriocins [35].

The fermentation pattern of silages after 7 days of air exposure are presented in Table 3. The DM, YM population, Tmax, HTmax, and pH were not affected ($p > 0.05$) by treatments and averaged 266 g/kg, 9 log cfu/g fresh weight, 40.7 °C, 69.1 h, and 6.20, respectively. Treatments also did not affect ($p > 0.05$) the production of lactic, acetic, and butyric acids and ethanol.

Table 3. Fermentation pattern, maximum temperature, and time to reach the maximum temperature of corn silage treated with *Lactobacillus buchneri* strains after 7 days of air exposure at 21 °C.

Treatment	Items								
	DM †	pH	YM ‡	Tmax ¶	HTmax ¥	LA €	AA ¾	BA £	Ethanol
CTRL	261	6.36	9.16	41.7	50.1	1.80	1.53	0.266	12.5
CI	252	6.61	9.11	41.8	67.9	2.25	3.26	0.233	9.23
LB-56.1	270	6.16	8.54	38.8	86.0	4.46	3.26	0.250	13.2
LB-56.2	264	6.15	8.46	40.7	88.1	2.85	1.86	0.233	16.3
LB-56.4	266	6.08	8.67	39.7	84.7	2.25	7.83	0.300	16.8
LB-56.7	265	6.24	8.76	40.8	69.1	1.35	2.20	0.150	18.3
LB-56.8	266	6.20	9.15	39.3	87.1	2.85	2.53	0.266	6.90
LB-56.9	269	6.00	8.90	39.7	80.4	2.60	2.40	0.200	14.8
LB-56.21	269	6.31	9.09	41.0	55.0	2.45	2.90	0.233	5.20
LB-56.22	274	6.07	9.00	39.8	62.6	1.66	2.63	0.266	15.9
LB-56.25	265	6.26	9.19	39.8	77.2	1.03	2.20	0.266	20.8
LB-56.26	266	6.25	8.94	41.6	57.3	1.70	2.26	0.233	22.6
LB-56.27	271	6.13	9.10	40.7	59.7	0.40	0.93	0.200	8.10
SEM	7.214	0.163	0.237	1.501	3.942	0.282	0.558	0.014	1.563
ANOVA *p*-value	0.98	0.19	0.34	0.87	0.09	0.09	0.60	0.85	0.27

† Dry matter (g/kg); ‡ Yeast and mold (log cfu/g of fresh weight); ¶ Maximum temperature (°C); ¥ Time to reach the maximum temperature (h); € Lactic acid (g/kg of DM); ¾Acetic acid (g/kg of DM); £ Butyric acid (g/kg of DM). CTRL: noninoculated; CI: commercial inoculant—Lalsil AS; LB-56.1-56.27—wild *Lactobacillus buchneri* strains.

The concentrations of propionic acid and 1,2-propanediol differed between treatments ($p < 0.05$) after 7 days of air exposure (Figure 3). Silages inoculated with LB-56.4 and LB-56.25 strains presented the highest 1,2-propanediol production, while the lowest value was observed in LB-56.27 silages. The strains LB-56.1 and LB-56.4 resulted in silages with higher propionic acid concentration than others (1.67 vs. 0.70 g/kg of DM).

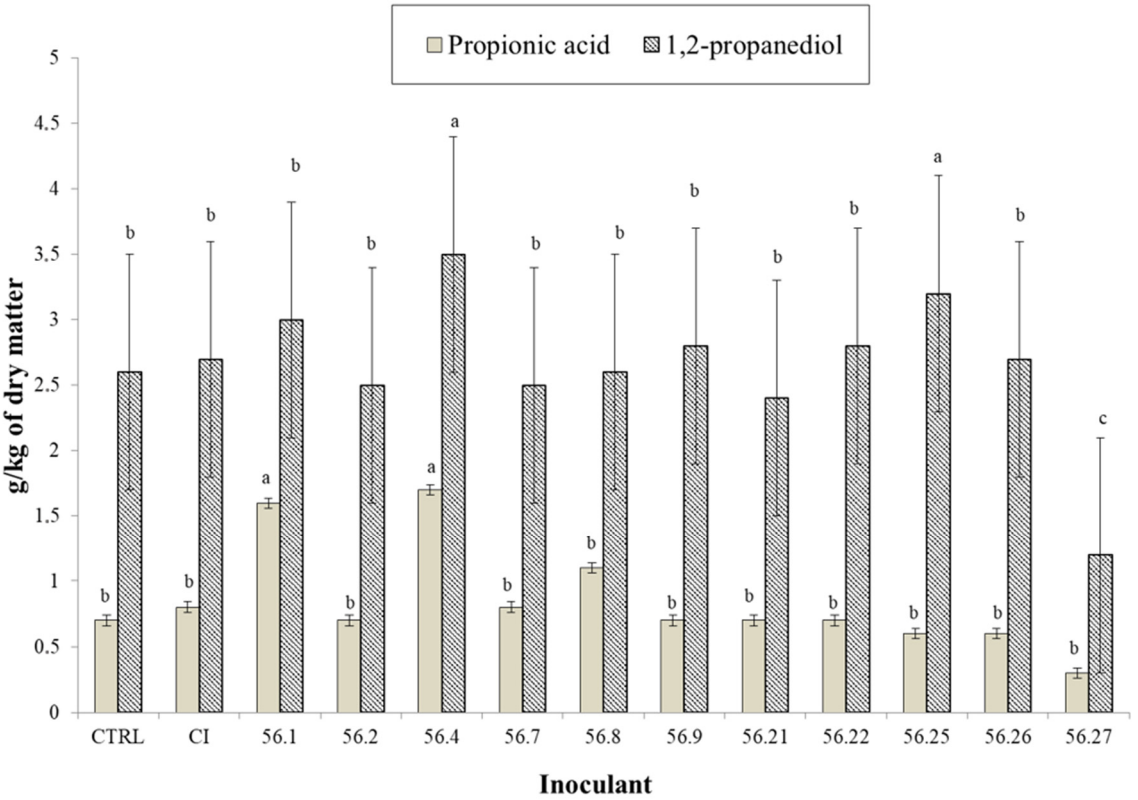

Figure 3. Propionic acid and 1,2-propanediol concentrations of corn silage treated with *Lactobacillus buchneri* strains after 7 days of air exposure at 21 °C. [a–c] Means within columns followed by the same letter group together according to the Scott–Knott clustering procedure ($p < 0.05$); Propionic acid—ANOVA p-value = < 0.01, SEM ± 0.08; 1,2-propanediol—ANOVA p-value = <0.01, SEM ± 0.18; CTRL: noninoculated; CI: commercial inoculant—Lalsil AS; LB-56.1-56.27—wild *Lactobacillus buchneri* strains.

After seven days of air exposure, all silages spoiled and had high YM population. The increased pH can be due to the reduction of organic acid concentrations by volatilization or utilization as substrate by yeasts, molds, or bacteria under aerobic conditions [36,37].

4. Conclusions

Inoculations of corn with the new strains of *L. buchneri* LB-56.1, LB-56.2, LB-56.4, LB-56.8, LB-56.9, and LB-56.25 provided higher aerobic stability than noninoculated silage, thus presenting feasible potential to be used as microbial inoculant in corn silage. The strain LB-56.1 resulted in silages 14 h more stable than the commercial inoculant. The evaluation of the new strains on silages is just beginning, and future studies will be carried out to evaluate and select the best growth conditions in different cultures to enhance the mode of action of these microorganisms for silage produced in tropical conditions.

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