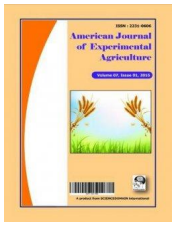

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Lactic Acid Bacteria of Potential as a Means of Inhibiting Undesirable Microorganisms in Warm Season Grass Silages

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Authors' contributions

This work was carried out in collaboration between authors. All authors read and approved the final manuscript.

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ABSTRACT

The presence of some microorganisms in silage besides reducing nutritional value and may represent risks to animal and human health due potentially pathogenic microorganisms. Enterobacteria, bacteria of the genus *Clostridium* spp and bacteria of the genus *Listeria* spp develop in badly fermented silage, in which pH drop is slower. After silos opening, yeasts, fungi and *Bacillus* spp initiate aerobic degradation, leading to pH rising and reappearing of *Clostridium* spp, *Listeria* spp and enterobacteria. Thus, development control those microorganisms by adequate fermentation is extremely important, since besides reducing silage quality, many are pathogenic or produce substances that are harmful to animal and human health.

Keywords: Bacillus; conservation; fungi; Listeria; pathogens.

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1. INTRODUCTION

Silage is a metabiosis, which means during the fermentative process a microbial succession occurs, that describes the different stages in silage process. Consequently, many groups of microorganisms develop simultaneously and in succession, as changes occur in redox and the type and amount of substrate [1,2].

Nutrient preservation in silage comes from fermentation by lactobacilli or other lactic acid bacteria (LAB). To obtain effective action from these microorganisms, four conditions are necessary: 1) fermenting material to allow bacterial growth; 2) oxygen absence in the material to favor the growth of anaerobic Lactobacilli; 3) enough number of Lactobacilli so that they are rapidly dominant over other microbial species; and 4) low humidity to avoid the produced acids to dilute favoring butyric fermentation [3,2,7].

The susceptibility of silage's deterioration seems to be ruled more by the fungal population than by the chemical composition of silage [4]. Aerobic microorganisms' breath may be considered one of the main agents that influence silage quality. However, the substrate used for breath depends on the type of microorganism, for example, yeasts consume only soluble chemicals (sugars and fermentation products), while molds degrade a large array of nutrients, including structural carbohydrates and lignin [1,5,6].

The presence of fungi is undesired, not only because they break the sugar and lactic acid by normal breath, but also for they hydrolyze and metabolize cellulose and other cellular wall components. Besides, some molds, mainly the species of the genus *Aspergillus*, *Fusarium* and *Penicillum*, grow in silage where there is air penetration and produce toxins that are harmful to animals and humans [2,7,18].

The best model for fermentative process is the one in which the lactic bacteria become dominant over the groups of undesired microorganisms.

Thus, the object of this review is to describe the effects of inoculation of lactic bacteria over the microbiological quality and silage stability, considering the importance and control of each microbial group involved in the silage process separately.

2. DEVELOPMENT

2.1 Enterobacteria

Enterobacteria are the group of microorganisms most widely studied. Among the reasons, stands out the medical importance and economic impacts, how easy they are to isolate and grow, rapid breeding time and easy genetic manipulation. They are found in the water, on the ground, in animals and humans' intestines and many vegetal tissues [1].

Enterobacteria are Gram-negative, oxidase-negative, do not grow spores, are shaped into short bacilli (0.3-1.0 x 1.0-6.0 p.m.). They move by peritrich flagello. They are not halophyilic and are facultative anaerobic. They are chemoorganotrophic and show respiratory and fermentative metabolism, growing well in temperatures between 22 and 35ºC. They are catalase-positive and reduce nitrate to nitrite [2].

Enterobacteria are divided according to the fermentation end products, mixed acid production, but anediol and tri-methylene glycol producers. Glucose catabolism occurs both by EMP (Embden-Meyerhof-Parnas), in which energetic yield of glycolytic way occurs, metabolic Phospho-dihydroxy-Acetone way and pyruvic acid metabolic way, as by HMP. *Escherichia coli*, Serratia, *Salmonella*, Klebsiellia, Aerobacter, Paracolobacterium, Erwinia, Proteus have HMP way identified already [5].

In silage, many microorganisms are undesired for two reasons: first due to pathogenicity, and since they will be used as animal food, these microorganisms should be absent in silage; second, the development of these bacteria results in great nutrient losses, since the metabolic was employed result in high substrate consumption which is lost in the form of secondary metabolites [1].

Studies have shown that such bacteria develops at the beginning of silage process and have their numbers reduced as the pH decreases. Usually their population decreases as lactic bacteria population growth [8]. Verified, in silage of *Digitaria eriantha*, that the enterobacteria population reached its maximum level in the first 24 hours of fermentation with values of 7.1 log of colony formation unit (cfu)/g, reducing to 6.3 log of colony formation unit (cfu)/g at 9 days of fermentation. [9], assessing microbial populations in corn silage, observed

enterobacteria values varying from 5.1 to 3.9 log cfu/g from the beginning to the tenth day of fermentation.

Penteado et al. [10] observed in guinea grass (*Panicum maximum* Jacq. cultivar Mombasa) silage increase of lactic acid bacteria population and the decrease of enterobacteria occurred in the first days of fermentation, showing that microbial succession occurs very rapidly and in a very definite way. However, the enterobacteria are still present in silage, despite the lower pH.

The explanations for microbacteria population reduction as the lactic bacteria increase is the simple reduction of pH or, as it has been shown by some authors, the production of bacteriocins. In Fig. 1. Can observe that the populations of enterobacteria decrease as the pH gets lower in guinea grasssilage and that such decrease is more severe in inoculated silage.

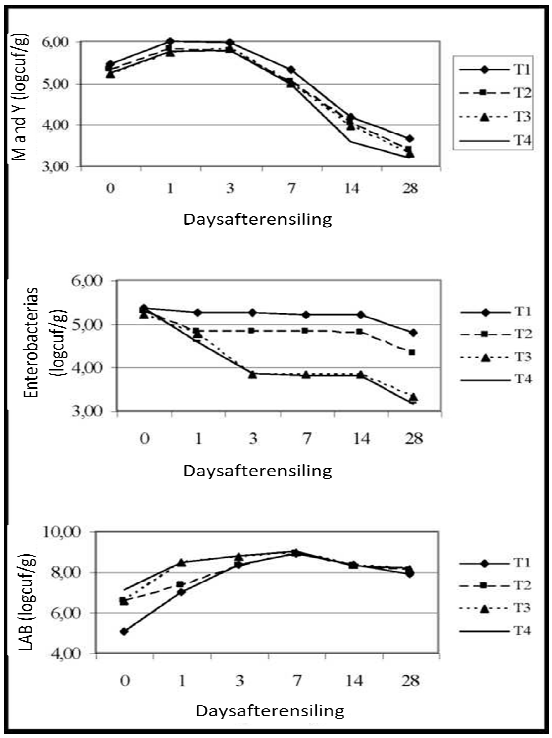

Fig. 1. Populations of molds and yeasts (M and Y), enterobacteria and lactic acidbacteria (LB), in mombasa grass silage without inoculant (T1), inoculated with 10^4CFU/g forage (T2), inoculated with 10^5CFU/g forage (T3) and inoculated with 10^6CFU/g forage of *Lactobacillus plantarum* of epiphytic microbiota

Adapted from [10]

Muck et al. [2] observed a reduction in ammonia concentration and in the population of enterobacteria in guinea grass silage (*Panicum maximum*) inoculated with *Lactobacillus plantarum*, isolated from the epiphytic microflora. Thus the inoculants for silage can facilitate or accelerate the process of ensiling, but they do not replace the fundamental factors (maturity of the plant, dry matter content, exclusion of oxygen), which are essential for producing good quality silage. Among these factors the age of regrowth is the one that influences all the features of the silage, from the fermentation of the silage to the nutritional value, considering the losses.

Penteado et al. [10] evaluated the aerobic stability of *Panicum maximum* cv. Mombasa silage inoculated with two strains of *Lactobacillus buchneri*, one from a commercial inoculant and another isolated from sugar cane (*Saccharum officinarum* L.) silage. It was observed an increase in dry matter content after silo opening, while the carbohydrate ratio did not change due to the low residual concentration, characteristic of grass silage.

Many LAB have antimicrobial peptides, known as bacteriocins, which are responsible for inhibiting growth of related species or species that have similar nutritional requirements. Consequently, bacteriocin production is a form of competition between bacteria that live in a same ecologic niche [2].

Bacteriocin production is a process that consumes a high amount of energy, therefore it is only worthy for the producing microorganism if really necessary. It is known that biosynthesis energy cost is high and, for that reason, it is a process well controlled by molecular regulatory systems with instantaneous catabolic induction and repression. Bacteriocin production is influenced by pH, temperature, environment composition, agents that damage DNA or growth conditions [11].

Antimicrobial activity performed by *Lactobacillus plantarum* was determined by [11,12], who purified and characterized a bacteriocin composed by two peptides, conducted by *Lactococcus lactis* and *Pediococcus pentosaceus*, which was named plantaricin NC8. [13]. Verified that *Lactobacillus* plantarum isolated in cassava (*Manihot esculenta* Grantz) and corn (*Zea mays*) produced a bacteriocin that has shown itself effective in inhibiting bacteria

from several geni, including *Staphylococcus*, *Bacillus*, *Clostridium*, *Listeria*, *Salmonella*, *Escherichia coli*, besides some lactic bacteria, such as *Streptococcus thermophillus* and *Leuconostoc mesenterioides*.

Silva [14] evaluating the antagonist effect of *Lactobacillus plantarum* isolated from corn verified that there was inhibition of *Eschecrichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

In corn and sorghum silage, [15] showed that enterobacteria do not survive to the end of silage process, fact that could be explained by the high LAB, as well as the rapid and steep drop in silage pH. While [10] verified occurrence of enterobacteria up to the eight fermentation day in mombasa grass silage (Fig. 1).

Thus, in corn and sorghum, inoculation is unnecessary in enterobacteria control, since such do not survive to the low resulting pH at the end of silage process. However, in grass silage, in which many times pH does not reduce enough to eliminate enterobacteria population, the inoculation is necessary and has shown itself effective in controlling enterobacteria growth [8,16,10,17,6,18].

However, [7] observed that the use of inoculant did not improve quality, nutritional and fermentation characteristics of silages *Panicum maximum* cv. Tanzania and cv. Mombasa [6] Reported that there was no effect on the pH value, ammonia, effluent production and recovery rate of MS in Tanzania grass silage, with and without the addition of in oculant.

In Table 1 can be observed the effects of inoculation with *Streptococcus bovis* (strains HC5 and JB1) isolated from rumen, homofermentative lactic bacteria about the development of lactic bacteria and enterobacteria in *Panicum maximum* cv. Mombasa silage. It was observed that in inoculated silage, there was larger development of lactic acidbacteria than enterobacteria. It is important to notice also the persistence of enterobacteria in silage until the twenty eighth day of fermentation, which shows how hard it is to eliminate this group of bacteria in grass silage [17,19,6,18].

2.2 *Clostridium* spp

Bacteria whose final fermentation products are acetate, butyrate, acetone and isopropanol. They are divided into saccharolytic (*Clostridium tyrobutiricum*), that produce butyric acid from sugar fermentation and lactic acid and proteolytic (*Clostridium sporogenes*), that degrade aminoacids, creating ammonia and amines and saccharo-proteolytic (*Clostridium perfringens*), that promote both fermentation and proteolysis [20].

Clostridium forms acetyl CoA or pyruvate acetyl phosphate, with the formation of carbon dioxide and hydrogen, without format (Transferase acetyl phosphate), while enterobacteria use format (hydrogen format lyase).

One of the main problems of the presence of *Clostridium* bacteria in silage is milk contamination. *Clostridium* spores are resistant to cooking temperature of certain cheese, developing and promoting undesired fermentation. The most important are *Clostridium tyrobutiricum* and *Clostridium sporogenes*. *Clostridium tyrobutiricum* ferments lactic acid into butyric acid and CO2 and *Clostridium sporogens* are proteolytic, degrading aminoacids and forming ammonia and amines (histamin, putrescin, cadaverin), which results in putrid smell in cheese [21].

According to [20] the presence of *Clostridium* spp is conditioned to buffering power of ensiled material, since this group of bacteria does not develop in very acidic environments. Thus, in corn and sorghum silage, such bacteria are eliminated by the sudden and extreme pH reduction and, consequently, there is a low accumulation of butyric acid and ammonia in silage.

As for grass silage, the younger and larger amount of humidity, the lower level of soluble carbohydrates, high buffering capacity, more likely the environment will be for the development of bacteria from this genus. For legume, besides the low amount of carbohydrates, the high protein level favors proteolysis, which results in high ammonia production and, consequently, silage with high pH. Such conditions favor butyric fermentation instead of lactic acid [8,9].

As happens with enterobacteria, *Clostridium* spp. bacteria can be inhibited also by the production of bacteriocins. [22] verified that a strain of Streptococcus bovis, called HC5 produces a bacteriocin (bovicin HC5) that inhibits a wide range of microorganisms, including bacteria from the genus Clostridium. Effective bacteriocin when inhibiting bacteria of this genus was also observed in *Lactobacillus plantarum* by [13].

Table 1. Average values of lactic acidbacteria (LAB), enterobacteria (ENT) for treatments throughout the fermentation period of mombasa grass silage

	Openingtime				
Treatments	**0**	**1**	**7**	**14**	**28**
	LAB, CFU/g				
Control	5.39Be	7.48Bd	8.85Aa	8.71Cb	8.27Bc
HC5	5.72Ae	7.81Ad	8.87Ab	9.11Ba	8.57Ac
JB1	5.77Ae	7.82Ad	8.94Ab	9.40Aa	8.69Ac
	ENT, CFU/g				
Control	6.01Aa	5.83Ab	5.94Aa	4.94Ac	4.29Ac
HC5	5.99Aa	5.58Bb	5.08Bc	4.57Bd	3.59Be
JB1	5.93Aa	5.48Bb	4.91Cc	4.53Bd	3.57Be

Averages followed byunlike capital letter in columns and same lowercase letter in rows differ by the Student Newman Keuls test; at 5% significance. Adapted from [17]

Thus, *Streptococcus bovis* inoculation is intended to reduce the development of bacteria from the genus *Clostridium* spp in grass and legume silage, reducing the formation of ammonia, amines and butyric acid and resulting in better quality silage. Besides, the inhibition of the development of this group of bacteria reduces the risks of milk contamination, keeping, thus, the quality of dairy products.

In Table 2 shows the development of lactic acid bacteria and from the genus *Clostridium* spp and the pH of *Digitaria eriantha* silage throughout the period of fermentation inoculated or not with inoculant containing *Enterococcus faecium*. Inoculation resulted in faster and more extreme pH drop, followed by larger development of lactic acid bacteria and lower development of bacteria from the genus *Clostridium* spp. [8].

In Table 3 can be observed the highest concentration of crude protein were observed in silages treated with *Streptococcus bovis* and HC5 and JB1. This may have been associated to the fact that the *Streptococcus bovis* HC5 species releases (bovicine HC5) bacteriocin in themedium that inhibits growth of proteolytic bacteria, such as the enterbacteria or clostridia, and thus decreases the protein nitrogen losses from the inoculated silages [18]. Another fact that may explain the greater concentration of CP in the silages inoculated with *Streptococcus bovis* HC5 and JB1 strains may reflect the common capacity of all the *Streptococcus bovis* strains to synthesize protein from ammonia [22,6].

2.3 *Listeria* spp

The genus *Listeria* spp contains Gram-positive bacillis, non-spores formers, mobile, catalase positive, and are facultative anaerobic. From the seven species found, two are majorly important due its pathogenic effect: *Listeria monocytogenes*, in animals and humans and *Listeria ivanovii*, in animals [23].

Listeriosis is an infectious disease caused by *Listeria monocytogenes*. It affects several animal species, inducing three forms of clinical manifestation: (1) sepsis with abscesses in viscera such as liver and spleen, (2) miscarriage and (3) neurologic disease (meningoencephalitis). The disease is more common in temperate weather regions, where cases occur mostly during winter and beginning of spring. Bad quality silage (pH above 5.5) favors the grown of these bacteria [24,27,28].

Table 2. Values of pH, population of lactic acid bacteria (LAB) and population of bacteria of the genus *Clostridium* spp (CL) in *Digitaria eriantha* with or without inoculant

Period of fermentation	***pH***	***LAB Log CFU/g***	***CL Log CFU/g***
0	6.00	1.60	0.70
1	6.30	5.80	0.50
5	6.10	7.10	1.05
9	5.70	7.20	1.90
Inoculated			
0	6.00	3.70	1.10
1	5.30	7.70	0.40
5	4.40	8.20	0.20
9	4.30	8.10	0.40

Adapted from [8]

Table 3. Average valuesof pH, ammoniacal nitrogen (N-NH_3) and concentration of dry matter (DM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF) and acid detergent fiber (ADF) in elephant grass silages without inoculant (control) and inoculated with *Enterococcus* (*Enterococcus faecium*), JB1 (*Streptococcus bovis* JB1) and HC5 (*Streptococcus bovis* HC5)

Treatment	pH	NH_3 (mg/dL)	DM (%)	CP (%MS)	EE (%MS)	NDF (%MS)	ADF (%MS)
Control	4.32a	11.44a	25.53b	6.23b	3.02a	69.74a	38.75a
Enterococcus	4.19b	11.09b	27.12a	6.30b	2.99a	69.37a	37.30a
Streptococcus bovis JB1	3.99c	10.54c	28.12a	6.98a	2.95a	71.71a	36.88a
Streptococcus bovis HC5	4.04c	10.68c	26.93a	7.08a	3.06a	68.09a	37.57a
CV (%)	2.02	2.12	3.87	3.58	5.78	6.76	5.97

Means within a column with unlike lettercase differ by the Tukey test at the level of 5% significance. Adapted from: [7]

As well as enterobacteria and bacteria from genus *Clostridium* spp and *Listeria* spp. develop better in silage with higher pH and is inhibited in silage with lower pH. [25] verified the presence of *Listeria* spp in 65.6% of the samples at the moment of silage opening of Cynodon sp (tifton-85) and, among them, 10% tested positive for *Listeria monocytogenes*, and all of the assessed silage showed pH levels above 4.70.

Evaluating the occurrence of different ribotypes of *Listeria* spp in corn and grass silage, [24] verified the presence of *Listeria* spp. in 10% of the corn silage samples and 60% in the grass silage samples, showing that grass silage are more susceptible to occurrence of *Listeria* spp. Besides, it was also verified that 83% of the isolates of *Listeria* spp in high quality corn silage were identified as *Listeria monocytogenes*, alerting for the presence of this pathogenic species even in high quality silage such as corn.

Besides the importance of adequate fermentation in controlling *Listeria* in silage, as described for enterobacteria and *Clostridium* bacteria, *Listeria* can be inhibited by LAB that produce bacteriocin, as demonstrated by [26]. In Table 4 There is observed that among the many species inhibited by LAB, to be included *Listeria monocytogenes*. *Listeria* inhibition was also observed by [22], evaluating the effects of bovicine bacteriocin HC% produced by *Streptococcus bovis* HC5.

2.4 Fungi

By the opening silos, occur the oxygen penetration, so aerobic bacteria, fungi and yeasts develop. Such microorganisms use residual sugars and some yeasts and acetic acid bacteria use lactic acid present in the environment, altering the redox potential promoting increased pH. Such conditions favor the reappearance of enterobacteria, *Listeria* spp and *Clostridium* spp., harming even more the microbiological quality silage.

Among the species that develop after silo opening, the fungi are extremely undesired, since, besides causing losses, like microorganisms are mycotoxin producers that can cause harms to animals' health and, in cases of higher intoxication, can be found in animal origin products and this represent risks to human health [8].

In Table 5 are summarized the main mycotoxins found in food and their effects over animal performance.

According to [20] fungi develop at the beginning of fermentative process, using the remaining oxygen among the plant particles and normally reach their maximum value at the first days of fermentation. Such microorganisms produce large amount of spores that are activated when silage is exposed to air by silo opening.

The high residual content of soluble carbohydrates in silage, mainly the ones made of corn, sorghum and sugarcane, favors the aerobic deterioration process by fungi and yeasts, causing losses after the silo opening. However, the organic acids produced by fermentation, mainly acetic acid, have fungicidal effect and can mitigate the deterioration, increasing aerobic stability of the silages [29,30,31,32].

Table 4. Antagonist activity (inhibition halo diameter) of *Lactobacillus* spp. and *Lactococcus* ssp. isolated in cheese

	Producer				
Revealer	***Lactobacillus casei***	***Lactobacillus fermentum***	***Lactobacillus rhamnosus***	***Lactobacillus acidophilus***	***Lactococcus lactis***
Bacillus cerens	87.25	90.00	90.00	71.64	36.84
Staphylococus aureus	51.88	49.54	52.61	33.17	20.16
Salmonella enteric	45.30	43.40	47.24	30.88	22.93
Yersinia enterocolytic	35.33	37.39	46.50	33.73	25.09
Listeria monocytogenes	35.03	47.07	44.80	39.14	22.81
Salmonella enteric	44.98	41.89	39.87	32.61	26.07
Shigella flexneri	79.32	84.18	86.22	90.00	63.60
Pseudonomas aeruginosa	89.12	81.92	86.12	34.64	74.60
Escherichia coli	54.62	41.17	69.77	28.04	54.87

Adapted from: [26]

Different from the positive results about enterobacteria control, *Clostridium* and *Listeria* spp bacteria, inoculants based on homofermentative bacteria are not effective in improving silage stability. In this case, heterofermentative bacteria are more effective, besides reducing the amount of lactic acid and residual carbohydrates in silage, producing more acetic acid inhibiting the development of fungi, yeasts and aerobic bacteria [8,9].

In Table 6 we can observe that in corn and sorghum silage, the development of fungi and yeasts, 5 days after silo opening was higher in silage inoculated with *Lactobacillus plantarum*, while inoculation with *Lactobacillus buchneri* that inhibited such microorganisms growth [27].

[28] also verified in corn silage that the inoculation with *Lactobacillus buchneri* inhibited the development of yeasts and increased aerobic stability of the silage. On the other hand, higher values of pH and N-NH3 were recorded, showing that yeast control and increase of aerobic stability by means of inoculation with heterofermentative bacteria may occur at the costs of some harmful effects over fermentative parameters.

Another way to improve aerobic stability of the silage is the use of propionic bacteria, which have the ability to transform three mols of lactate into two mols of propionate, one mol of acetate and one mol of CO2. [29] verified that the inoculation of *Propionibacterium* acidipropionici increased the amount of propionic acid and acetic acid in corn, sorghum and hay silage and decreased the amount of CO2 produced after silo opening, presenting itself effective in improving stability of such silage.

Considering the aspects of aerobic instability, could conclude that in the composition of certain inoculant for corn and sorghum silage, or other forage species with high level of soluble carbohydrates, there may be present heterofermentative lactic bacteria or propionic bacteria, thus ensuring aerobic stability of silage after silo opening.

Table 5. Mycotoxin most common effects in food over animal performance

Mycotoxins	**Effects on animals**
Aflatoxin	Liver compromising
Ochratoxin	Weight loss
Deoxynivalenol	Low consumption,food refusing
Thicothene T-2	Low consumption,food refusing
ZearalenoneF-2	reproductive disorders
Slaframin	Diarrhea

Adapted from: [21]

Table 6. Fermentative profile and dry matter loss in silage of corn and sorghum without inoculant (C) or inoculated with *Lactobacillus plantarum* (LP) or *Lactobacillus buchneri* (LB)

		%						
		pH	SC*	Acid lactic	Acid acetic	Ethanol	N-NH3	DM losses**
Corn	C	3,72b	3,15a	4,04c	1,27b	0.47	0,26b	1,65b
	LB	4,13a	0,64b	2,76d	3,89a	0.49	0,28a	3,26a
	LP	3,64b	2,54a	7,94a	0,33c	0.42	0,21c	0,75c
	LB + LP	3,80a	1,08b	5,55b	3,17a	0.45	0,22c	1,14bc
Sorghum	C	3,87b	6,75a	4,86a	0,96b	0.50	0,28b	1,97b
	LB	4,26a	1,36b	2,54d	4,30a	0.53	0,30a	3,49a
	LP	3,75b	5,96a	9,39a	0,62c	0.47	0,24c	0,94c
	LB + LP	3,88b	2,02b	6,18b	3,49a	0.49	0,24c	1,45bc

**Soluble carbohydrates; ** dry matter losses; Adapted from: [27]*

Table 7. Carbohydrate fermentation profile of the isolates EB1, EB2, EB5, and EB6, signal grass plants (*Brachiaria decumbens* cv. Basiliski). $^{+}$ Intense fermentation, $^{-}$ no fermentation; $^{(+)}$ less intense fermentation

	Isolated strain				*Lactobacillus plantarum*
	EB1	EB2	EB5	EB6	
Glycerol	-	-	-	-	-
Erythritol	(+)	(+)	(+)	(+)	-
D-arabinose	-	-	-	-	-
L-arabinose	+	+	+	+	+
Ribose	+	+	+	+	+
D-xylose	-	-	-	-	-
L-xylose	-	-	-	-	-
Adonitol	-	-	-	-	-
β-methyl D-xyloside	-	-	-	-	-
Galactose	+	+	+	+	+
D-glucose	+	+	+	+	+
D-frutose	+	+	+	+	+
D-mannose	+	+	+	+	+
L-sorbose	-	-	-	+	-
Rhamnose	(+)	(+)	(+)	(+)	-
Dulcitol	-	-	-	-	-
Inositol	-	-	-	-	-
Mannitol	+	+	+	+	+
Sorbitol	+	+	+	+	+
α-methyl D-mannose	-	-	-	-	+
α-methyl D-glycoside	-	-	-	-	-
N-acetyl-glucosamine	+	+	+	+	+
Amygdaline	+	+	+	+	+
Arbulin	+	+	+	+	+
Esculin	+	+	+	+	+
Salicin	+	+	+	+	+
Cellobiose	+	+	+	+	+
Maltose	+	+	+	+	+
Lactose	+	+	+	+	+
Melibiose	+	+	+	+	+
Saccharose	+	+	+	+	+
Trehalose	+	+	+	+	+
Inulin	-	-	-	-	-
Melezitose	+	+	+	+	+
D-raffinose	+	+	+	+	+

	Isolated strain				*Lactobacillus plantarum*
	EB1	**EB2**	**EB5**	**EB6**	
Amidon	-	-	-	-	-
Glycogene	-	-	-	-	-
Xylitol	-	-	-	-	-
β-gentibiose	+	+	+	+	+
D-turanose	+	+	+	+	+
L-lyxose	-	-	-	-	-
D-tagatose	-	-	-	-	-
D-fucose	-	-	-	-	-
L-fucose	-	-	-	-	-
D-arabitol	(+)	(+)	(+)	(+)	-
L-arabitol	-	-	-	-	-
Gluconate	+	+	+	+	+
2 Cetogluconate	-	-	-	-	-
5 Cetogluconate	-	-	-	-	-

Adapted from: [30]

Kung et al. [30] conducted a study aiming to characterize and quantify microbial populations in signal grass, harvested at different ages of regrowth. The six strains of lactic acid bacteria isolated from the signal grass were characterized according to the Gram-staining, catalase enzyme reaction, and the form of bacilli, submitted to tests for growth and identification. The identification of the isolates was performed by fermentation of carbohydrates in kit API 50 CH (Bio Meurix-France) (Table 7 above).

3. CONCLUSION

The control of undesirable microorganisms like enterobacteria, *Clostridium* spp. and *Listeria* spp. are performed by adequate fermentation, so that corn and sorghum silage, as well as silage inoculated with homofermentative lactic bacteria are less willing to the development of such microorganisms.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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