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Potential use of native microorganisms strains of forage for silage production

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Introduction

Ensilage is the name given to the process of conserving forage plants or other products through fermentation. This process is characterized by fermentation, mainly by lactic acid bacteria (LAB) from carbohydrates present in the original material that primarily produce lactic acid as well as other acids that lower pH and conserve the ensiled forage.

The quality of this fermentation is influenced by the type and quantity of the acids formed, which, in turn, depend on the conditions under which the silage was produced, and especially by the microorganism population present throughout the process. Microorganisms are beneficial when they produce acids and lower pH.

However, they can also be harmful when they break down proteins, produce compounds that are toxic for animals or even man, or just compete with beneficial microorganisms for substrates.

Silage fermentation may occur naturally due to the epiphytic microorganisms present in the plant to be ensiled or added microorganisms to improve the silage process and quality. Microorganisms added at the beginning of the fermentation process are called inoculants or culture starters.

Inoculant adequacy for individual forage types is measured by fermentation improvement. Although all inoculants share some general features, individual forage characteristics and silage conditions may require specific inoculants to improve the fermentation. The purpose of the present work is to discuss bacteria selection for silage inoculation with an emphasis on native tropical forage bacteria strains.

Selection of silage inoculants

Bacterial inoculants are microbial additives used in the fermenting process to improve the quality of the final product. Several biotechnological processes related to fermented beverages, meat, yogurt, and probiotics among others are concerned with selecting the most efficient microorganisms. In general, these industrial processes are easy to control; however, the same cannot be said for silage. The main problems in silage production are difficulty in controlling fermentation on a large scale, ease of contamination and impossibility of interfering in the process during fermentation.

Lactic acid bacteria (LAB) stand out in microorganism selection for silage inoculation. These bacteria can be homofermentative or heterofermentative. Both bacteria types are potentially beneficial to the silage process. Homofermentive bacteria primarily decrease pH levels, inhibit undesirable microorganism growth and improve fermentation quality (Cai et al., 1999). Heterofermentive bacteria, on the other hand, produce lactic, acetic and/or propionic acids (Axellson, 1998), which are more effective in inhibiting fungal growth and subsequently improving stability of silage exposed to the air (Driehuis et al., 2001; Filya, 2003).

There is a wide variety of commercial silage inoculants. The species most commonly used belong to the genera *Lactobacillus (L. plantarum, L. acidophilus, L. buchneri)*, *Enterococcus (E. faecium)*, and *Pediococcus (P. acidilactici)*, as well as propionic acid (*Propionibacterium acidipropionici*) bacteria. Many studies describe advantages of these silage inoculants (Kleinschmit et al., 2006; Zopollatto et al., 2009). Despite the epiphytic LAB population being able to transform soluble carbohydrates into organic acids and lower the pH, this population is generally small and produces lower quality silage. Although studies generally demonstrate that the use of inoculants provide some benefits, results are contradictory when different forage cultures are evaluated under diverse conditions. Researchers have come to the following conclusion: the positive effect of inoculants on the fermentation process or silage quality depends on several factors related to forage culture and ensiling conditions.

The following should be considered during selection of silage bacterial strains: microorganism origin, characterization and identification; definition of pre-selection characteristics; microorganism evaluation on a small (laboratory) and large (farm) scale as well as evaluation of different cultures.

Effect of microorganism origin on selection

The origin of microorganism strains considered for selection may be different. According to Muck (2008), a determining factor in success of silage microbial inoculants is plant-microorganism compatibility. Forage crops have diverse chemical compositions and can store different types and concentrations of carbohydrates. These factors associated with the environment where a plant grows interfere in microorganisms' adaptation to a culture. Thus, it is believed that when a microorganism is selected to act on a specific forage plant from which it was selected, its adaptation to the species will improve performance during fermentation. Hill (1989) isolated three *Lactobacillus plantarum* strains in corn (*Zea mays* L.), alfalfa (*Medicago sativa* L.) and sorghum (*Sorghum* sp) that were applied to their respective plant species during ensiling. This author found the dominant strain to be native to the silage plant in question.

Native plant strains are not necessarily more efficient than strains isolated from other environments. Strains selected from other environments or strains selected from different forage cultures can also perform well in the silage process of a specific forage culture. Ávila et al. (2010) evaluated characteristics of sugarcane (*Saccharum* spp.) silage inoculated with strains isolated from sugarcane itself and commercial strains isolated from other environments (Table 1). Results showed that strains isolated from sugarcane did not always present the best results. The lower ethanol levels were found across silages inoculated with strains isolated from sugarcane (*L. brevis* UFLA-65-SIL; *L. buchneri* UFLA-72-SIL) and commercial strains isolated from other environments (*L. plantarum* Biomax 5®; *L. buchneri* Silo MaxLalsilCana®).

Strains with "target" characteristics are commonly used in the selection process. In this case, strains with recognized activity against silage deteriorating microorganisms such as coliforms, endospore-forming bacteria (*Clostridium* and *Bacillus* genera), *Listeria* spp., yeast and filamentous fungi could be included. It is important to verify if these strains could survive under ensiling conditions, in other words in an environment with reduced concentration of oxygen and low pH. Saarisalo et al. (2007) had positive results regarding inoculation with strains from non-silage fermentation processes (barley gain, pickled cabbage, meat inoculums). These authors concluded that these strains were effective in improving fermentation.

It is important to stress that the effect does not depend solely on the specie. Although microorganisms from the same specie present similar characteristics, there are variations across strains. Ávila et al. (2010) and Saarisalo et al. (2007) found that inoculation with different strains that were, although belonging to the same species, also resulted in silage with different characteristics, which shows that studies should consider strains and not just species.

Several characteristics of silage inoculants has been described such as: rapid growth and the ability to compete with natural plant microbiology; intense production of lactic acid to quickly reduce pH; tolerance to acidic conditions; no pathogenic effect and survival throughout the silage fermentation process (McDonald et al.,

Table 1. Lactic acid, acetic acid, propionic acid, ethanol concentrations and lactic: acetic acid ratio in sugarcane silages treated with inoculants.

Silage	Lactic acid	Acetic acid	Lactic: acetic acid	Propionic acid	Ethanol
	(% of DM)				
Control, without inoculant	1.73 b	1.74 c	1.00 b	0.58 c	6.14 a
L. plantarum UFLA-1-SIL strain	3.02 a	1.75 c	1.76 a	0.16 d	3.44 b
L. paracasei UFLA-67-SIL strain	3.80 a	1.83 c	2.07 a	0.17 d	3.67 b
L. brevis UFLA-65-SIL strain	2.10 a	4.97 a	0.43 b	0.12 d	1.15 e
L. buchneri UFLA-72-SIL strain	3.06 a	4.07 a	0.75 b	1.34 a	2.10 d
L. buchneri Pioneer 11A44TM	3.17 a	4.30 a	0.73 b	0.71 b	2.85 c
L. plantarum Biomax 5®	2.58 a	3.17 b	0.79 b	0.16 d	2.05 d
L. buchneri Silo MaxLalsilCana®	3.03 a	4.55 a	0.66 b	0.16 d	2.15 d
Average	2.81	3.30	1.03	0.44	2.94
Coefficient of variation (%)	18.9	12.6	20.9	16.3	10.9
Contrasts					
Control vs others[2]	**	**	*	**	**
L. buchneri vs *L. plantarum*[3]	ns	**	**	**	*
L. brevis vs *L. buchneri*[4]	*	*	ns	**	**
L. buchneri vs SiloMaxLalsilCana®[5]	ns	ns	ns	**	ns

Application rate of 10^5 ufc/g of forage.
[a,b] Means followed by the same letter in a column do not significantly differ from each other by the Scott-Knott test (P>0.05).
[2]Contrast between silage not inoculated vs inoculated; [3]Silage inoculated with *L. buchneri* vs *L. plantarum*; [4]Silage inoculated with *L. brevis* vs *L. buchneri*; [5]Silage inoculated with *L. buchneri* isolated from sugar cane vs commercial inoculants containing this same species.
ns: not significant; *(P<0.05); **(P<0.01).

1991; Saarisalo et al., 2007). A strain rarely presents all of the above characteristics. Consequently, the choosed forage to be ensiled should be analyzed. The forage fermentation process should be known as should the microorganisms involved. In this way, possible silage problems can be identified and specific microorganisms can be selected.

Criteria for inoculant selection

Substrate use and metabolite production

Different forage plants have different substrates available for bacteria (inoculants) to use. In temperate grasses, water soluble

carbohydrates mainly consist of fructan, sucrose, glucose, and fructose (McDonald et al., 1991). The main reserve carbohydrate in tropical forage is starch, with the exception of sugarcane which mainly accumulates sucrose (Van Soest, 1994). Some LAB are capable of using starch; however, most use simple carbohydrates in their metabolism.

The silage fermentation is a very dynamic process. A wide variety of microorganisms are present during the fermentation. These microorganisms have varying abilities to use different substrates and that diversity may make the fermentation process more complicated. On the other hand, the ability of inoculants to use carbohydrates can be an advantage in the competition against other microorganisms, mainly when limited amounts of soluble carbohydrates are available (Saarisalo et al., 2007).

The bacteria's ability to use a specific carbohydrate depends on the presence of enzymes capable of breaking down the carbohydrate structure. Species and strains within the same species perform differently. Consequently, strains should be evaluated concerning growth rate, carbohydrate use and the quantity and quality of the metabolic products (acids) formed. The main or most abundant fermentation products are lactic acid, acetic acid and propionic acid, which are also the most important ones for silage conservation. Decreased pH is also important; however, this characteristic should not be used singularly, since it is generally related to lactic acid production. For this reason, heterofermentative strains that are important for acetic acid production and less able to lower pH should never be selected since they have an inverse relationship.

Figure 1 illustrates principle components analysis of metabolites produced during fermentation of LAB strains in sugarcane juice. Note that each group is correlated with the production of one of that evaluated metabolites (acetic acid, propionic acid, ethanol, methanol or lactic acid). Bacteria most highly correlated with acetic acid are in different quadrants that those correlated with lactic or propionic acids. This can be explained in function of homo- or heterofermentative metabolism and demonstrates that selection should be directed toward specific acid production (Figure 1).

Antimicrobial Activity

Many species or microorganism groups are considered undesirable during the ensiling process. These microorganisms can undergo proteolysis which reduces the nutritional value of silage such as enterobacteria and bacteria of the *Clostridium* genus; perform secondary fermentation, use the lactic acid produced and consequently hinder conservation such as clostridia and yeast; as well as produce toxic compounds or reduce silage acceptability to animals such as ammonia nitrogen, butyric acid, biogenic amines, bacterial toxins (enterbacteria and *Bacillus*) and mycotoxins produced by filamentous fungi. Many microorganisms (fungus, *Bacillus, Listeria*) are directly involved in the aerobic deterioration of silage. Furthermore, some microorganisms may be present that are

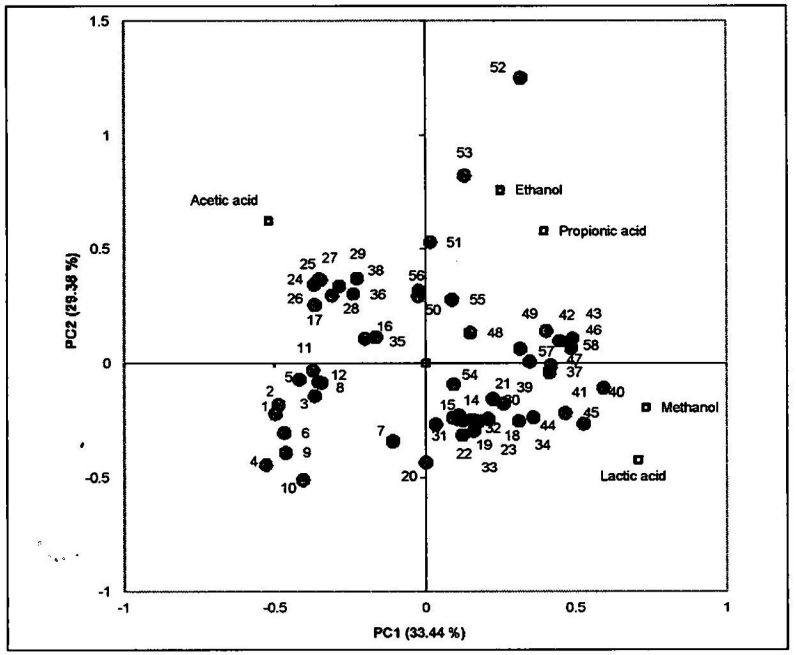

Figure 1. Principle components analysis of metabolites produced during fermentation by LAB strains in sugarcane juice (Ávila, 2010; unpublished data).

considered pathogenic to animals or man, such as *Listeria monocytogenes* species or *Clostridium* genus. LAB may potentially inhibit these microorganisms by producing acids or bacteriocins. The later are proteinaceous toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strains.

Studies have shown bacteriocins' potential to control growth of pathogenic microorganisms in food products. Bacteriocin producing *Lactobacillus* have emerged as protective cultures in fermented meat, olive and dairy food products (Hammes and Vogel, 1994; Leroy and De Vuyst, 2004). The ability to inhibit is inherent to each strain; however, evaluation of this capacity is interesting in a selection process.

Biogenic amine production

LAB strains may be able to produce biogenic amines. This production is linked to protein breakdown during ensiling and is formed by enzymatic decarboxylation of free amino acids and transamination of aldehydes and ketones (Nishino et al., 2007). The relationship of LAB with biogenic amine production may occur in two ways: LAB may be able to produce biogenic amines or they may inhibit microorganisms that produce these compounds. Either way, it is an important characteristic that should be evaluated when selecting bacterial strains for silage. Nishino et al. (2007) evaluated two strains of *Lactobacillus buchneri* and one of *L. casei* in different silages. The effects were variable depending on the strain and ensiled material. The *L. casei* strain inhibited biogenic amines in two silages, and the two *L buchneri* strains inhibited biogenic amines in only one silage type.

Due to the complexity of the ensiling process, it is difficult to determine which microorganisms are actually involved in biogenic amine production, since many microorganism genus such as *Bacillus, Klebsiella, Escherichia, Pseudomonas* and LAB (*Lactobacillus, Pediococcus* and *Streptcoccus)* can produce these substances (Santos, 1996).

Survival of bacterial strains during ensiling and tolerance to adverse conditions

In order for strains to survive during the silage fermentation process, they should be able to tolerate conditions that prevail in

the silo, such as high acidity and a low concentration of O_2. All LAB species grow anaerobically; however, most are not sensitive to oxygen and can grow in its presence (Madigan et al., 2010). LAB are generally resistant to acidic conditions, and lactobacilli are more resistant than other lactic acid bacteria. Regarding temperature, most LAB are mesophilic. In other words, they grow in moderate temperatures of about 35°C.

In general, ensiling conditions are conducive to LAB growth; however, many times these bacteria are exposed to high temperatures. This exposure may occur when inoculants are mixed with water in application tanks that are exposed to high temperatures (40 a 45°C). Furthermore, temperature increases may also occur throughout the fermentation process, which may reduce inoculant viability and efficiency in the silo. Due to Brazil's tropical climate, room temperature and that inside the silos can reach values above 45°C, which negatively affects viability of many inoculant strains. A bacterial strain's ability to resist a given stress factor may be key to inoculant success. Selection of strains tolerant to high temperatures is extremely important, as verified by Mulrooney and Kung Jr. (2008) and Cai et al. (1999), since the number of viable strains was greater when silage temperature remained within that for superior strain growth.

Viability of inoculant strains should be verified in order to determine if an inoculant strain can dominate the natural microflora and act throughout the entire fermentation process. On the other hand, verification is somewhat difficult. The most common method to determine strain viability in an environment is plating on culture media. Nevertheless, to know if an isolated strain is the same one that was inoculated, it needs to be identified, which can be time consuming. In this case, molecular techniques would be more interesting. Qualitative molecular techniques, in other words those that only identify species, cannot determine the microbial population present. Consequently, quantitative or real-time polymerase chain reaction (PCR) would be a better option. This technique can quantify a specific microbial species and associate results with qualitative data regarding fermentation, pH, production of organic acids, microbes, etc. (Schimidt et al., 2008).

Conditions concerning growth, lyphilization and storage are also important during large scale microorganisms' production. Freezing and lyophilization are commonly used in industrial inoculant production. During the lyophilization, freezing is detrimental to the cells. Strain viability after lyophilization depends on a microorganism's initial growth conditions. Factors like culture growth phase, temperature, microorganism concentration and protective additives may change microorganism viability; however, these conditions vary greatly from microorganism to microorganism (Morgan et al., 2006). As a result, several factors may influence cell viability after lyophilization, and these factors should be taken into account. Many studies have focused on understanding or improving resistance to bacterial strains. Cryoprotectants like glycerol, lactose, sucrose, trehalose, ascorbate and glutamate are usually added to protect cells from stress damage. Nevertheless, some microorganisms cannot tolerate lyophilization (Morgan et al., 2006). All these factors are very important for inoculant efficiency. Therefore, strain tolerance under different conditions should be evaluated.

Other characteristics evaluated

Inoculants have been reported to improve animal performance even when pH and acid production were not altered (Contreras-Gouveia, 2011). Consequently, these effects may be due to silage microorganisms in the rumen. According to Weinberg et al. (2004), inoculants may have a probiotic effect on ruminant performance, although the mechanism responsible for this is still not clear. LAB interaction with rumen microorganisms may improve rumen activity and fiber degradability. Another possibility is that LAB produces bacteriocins in silage, which might inhibit harmful microorganisms in both silage and rumen.

Little is known about how inoculants act during rumen fermentation. However, some studies indicate that inoculation with bacteria improve not only forage fermentation but also animal performance, as indicated by increased milk production, weight gain and/or feed intake (Contreras-Gouveia et al., 2011; Kung Jr. and Muck, 1997; Kung Jr. et al., 2003).

Other LAB characteristics that have already been explored by the food industry could also be studied for silage. LAB strains may be able to grow in the presence of phenolic compounds or use these substances which are secondary plant metabolites able to bind proteins. This quality is important for waste use in animal feed as well as fermentation during ensiling and in the rumen (Lopez-Guzman et al., 2009). An example would be inoculants used jointly with crude glycerin, which is a biodiesel waste product. According to Axellson (1998), some *Lactobacillus brevis* and *L. buchneri* strains can use glycerol as an electron acceptor in co-fermentation with glucose. The end products of this co-fermentation are lactate, acetate, CO_2, and 1,3-propanodiol. Dias Júnior et al. (2010) observed that glycerin used jointly with an inoculant containing a *L. buchneri* strain isolated from sugarcane silage resulted in sugarcane silage with less DM loss. This reduction was not observed when glycerin was used alone.

Some studies have shown that LAB can bind aflatoxins, which are one of the main mycotoxin groups produced in silage. This reduces its bioavailability or permits biotransformation into less toxic compounds (Niderkorn et al., 2007; Hernandez-Mendoza et al., 2009).

Results obtained with selection of bacterial strains for sugarcane and corn silage

The strain selection process for silage inoculation contains three main steps: pre-selection in laboratory, selection in lab silos and selection in farm silos. Pre-selection should be based on aforementioned characteristics, observations of the cultures to be ensiled, and fermentation problems. Studies conducted at the Federal University of Lavras (UFLA) have focused on selection of microorganisms isolated from its own silage to be used primarily to ensile sugarcane and more recently corn.

To proceed with selection, microorganisms should first be isolated and characterized. The strains isolated from sugarcane silage have been identified as belonging to the *Lactobacillus* genus. The primary species found were *Lactobacillus plantarum, L. buchneri, L.*

brevis (Ávila et al., 2009) and *L. paracasei* (Ávila et al., 2010). For pre-selection, strains are identified at least at a genus level, so that only bacteria from the LAB group are included. Pre-selection characteristics are different for each forage culture. In the present study, metabolite production and capacity for strain growth were evaluated in forage-like substrates, being sugarcane juice and corn extract.

Metabolite production is a leading characteristic in pre-selecting strains for ensiling sugarcane and corn. In a study to evaluate metabolite production of 58 strains isolated from sugarcane silage, metabolite production in sugarcane juice varied greatly across strains (Ávila, 2011, unpublished data). Production of lactic, acetic and propionic acids as well as methanol and ethanol respectively varied 0.88 to 8.5; 0.89 to 6.12; 0 to 0.46; 0 to 3.83 and 0 to 0.05 g/L. These differences are related to the strain's natural metabolism. Heterofermentative strains were generally the ones that produced the greatest amount of acetic acid, although some homofermentative strains also showed increased production of this acid compared to heterofermentative strains. This indicates that a greater concentration of acetic acid may also be related to other factors.

Results were submitted to principle components analysis (Figure 1). Strains were grouped according to the metabolites produced. The greatest producing strains of each evaluated acid were grouped into different quadrants. Strains correlated with propionic acid were also correlated with ethanol, and those correlated with lactic acid were also correlated with methanol. Some isolated strains were not correlated with any desirable metabolites for silage and were consequently discarded from the strain selection process. Higher lactic acid production is desired to efficiently reduce silage pH. Furthermore, acetic and propionic acids have been proven to be effective in controlling fungi. Regarding metabolism, greater lactic acid producing strains are not the greatest producers of acetic and propionic acids.

Based on these results, the best producing strains of each metabolite of interest were chosen and a new experiment was conducted in PVC silos to evaluate silage characteristics. Figure 1 presents the best producing strains of acetic acid (numbers 17, 24, 25, 27 and 35), lactic acid (numbers 17, 32, 33 and 34), and propionic acid

(numbers 41, 42, 46, 51 and 55). In the silo experiment, no differences were observed among LAB counts, yeast counts and silage pH values (Table 2). On the other hand, DM loss widely varied (Figure 2) throughout the fermentation process and among aerobic stability parameters (Table 3) (Carvalho, 2011 unpublished data).

DM loss during sugarcane ensiling is a great concern. Consequently, an inoculants' ability to reduce DM loss is one of the most important characteristics evaluated. In the present study, DM losses in silage generally increased with fermentation and varied greatly depending on the inoculant (Figure 2). There were no significant interactions among inoculant factors and fermentation time; however, there were differences among strains with average values varying from 11.38 to 22.90% (Table 3). It is interesting to correlate DM loss during ensiling with metabolite production in inoculated silage strains. All strains selected for greater lactic acid production did not improve (strain 33) or increase DM losses (19, 32 and 34) in silage compared to the control (Table 3). Among silage with less DM loss, two are better producers of acetic acid (17 and 24) and two are better producers of propionic acid (51 and 55). Results proved that this pre-selection methodology was efficient in sugar cane silage.

Table 2. Lactic acid bacteria (LAB) and yeast populations, pH values 4 d of air exposure in sugarcane silage inoculated with different LAB strains.

Variable	Duration of fermentation (days)			
	12	30	61	126
LAB (log ufc/g silage)	8.64 a	6.69 b	6.97 b	6.51 b
Yeast (log ufc/g silage)	5.77 ab	6.04 a	5.28 bc	5.00 c
pH	3.59 b	3.63 b	3.62 b	3.98 a

Means followed by the same letter in a row do not differ among themselves according to the Tukey test ($P > 0.05$).

Regarding aerobic stability, time (h) to lose stability was statistically different for the control (14.2 h) and silage inoculated with strain 55 (30.2 h). In other words, the inoculated silage was twice as stable. Silage temperatures after opening the silo were evaluated. Temperature of silage inoculated with strain 55 was always lower

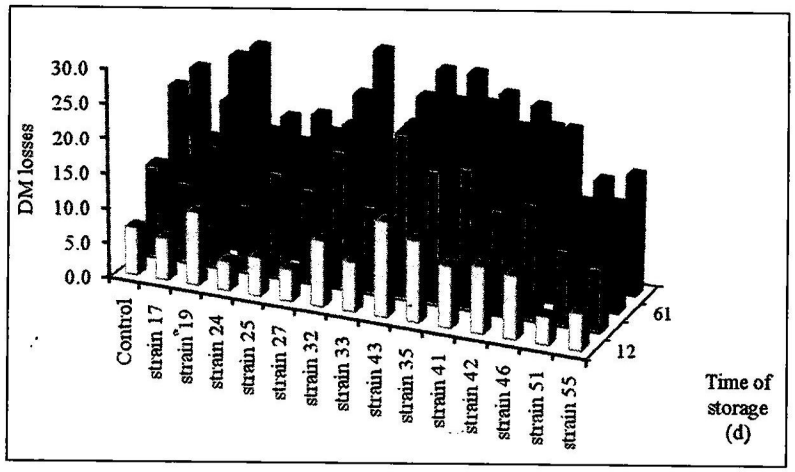

Figure 2. DM loss in sugarcane silage without inoculants (control) and inoculated with 14 different lactic acid bacteria strains (Carvalho, 2011 – unpublished data).

than the control. This strain stood out for decreasing DM loss and increasing aerobic stability, and was considered to have high potential for ensiling sugarcane.

Even though an inoculant may not influence the general population of some microbial group; it may act on some species and thereby modify fermentation quality. Often, this can also explain why no differences among LAB populations are detected while differences in silage quality are noted. Total LAB population counts did not indicate which species survived. As can be seen, different species present very different characteristics and consequently act differently in silage.

Another experiment was conducted to evaluate pre-selection and performance of some of these strains in corn silage. For this, some strains previously evaluated for sugarcane silage and other strains also isolated from this culture were grown in corn extract agar. After 48 h fermentation, samples were removed from the medium for metabolite analysis using high efficiency liquid chromatography (HELC), and results were submitted to principle components analysis (PCA) (Figure 3). In this experiment, a test was

Table 3. Total DM loss throughout 126 days of fermentation and aerobic exposure of sugarcane silage inoculated with LAB strains.

Silage*	Dry matter loss (%)	Maximum temperature (°C)	Time (h) to reach maximum temperature	Time (h) to lose stability (26°C)
Control	16.42c	47	22.0	14.2b
Strain 17	13.07d	46.3	28.8	18.0ab
Strain 19	20.93a	45.2	26.0	17ab
Strain 24	12.50d	45.0	33.3	18.2ab
Strain 25	14.19c	43.8	27.5	16.0b
Strain 27	13.89c	47.2	28.0	18.3ab
Strain 32	21.05a	43.5	42.2	20.2ab
Strain 33	14.57c	46.0	27.2	17.8ab
Strain 34	22.90a	43.0	48.2	21.0ab
Strain 35	21.38a	41.2	35.7	22.0ab
Strain 41	20.45a	43.8	30.3	19.8ab
Strain 42	18.40b	44.2	28.5	17.5ab
Strain 46	18.23b	43.2	27.5	17.8ab
Strain 51	11.38d	41.8	58.0	21.5ab
Strain 55	11.75d	43.8	54.0	30.2b

Averages followed by the same letter in a column do not differ statistically according to Scott-Knott test.
* Strain names represent codes used in figure 1(PCA).

included to evaluate the ability of strains to inhibit growth of deteriorating and pathogenic microorganism (*Escherichia coli* – ATCC 11229; *Clostridium perfringens* – ATCC 3624; *Bacillus cereus* – ATCC 11778 e *Listeria monocytogenes* – ATCC 19117) (Oliveira, 2011 - unpublished data).

Metabolite production in corn extract varied greatly among the strains evaluated. Strains were also grouped differently in this medium. Those that correlated with greater acetic acid also correlated highly with propionic acid. On the other hand, similar to growth in sugarcane agar, strains that correlated with lactic acid did not correlate with propionic and acetic acids (Figure 3). Most strains tested for growth in sugarcane agar (36 strains) were also tested in corn extract medium. Results varied between the two mediums. In other words, the greatest producers of a certain acid in sugarcane extract were not good at producing the same acid in corn extract. This indicates that selection should also differ. Selection should re-

flect a strain's capacity to use the substrates present in the different media (sugarcane or corn extract). Just as these strains act differently in the two mediums, they should act differently in silage.

Strains were ranked based on metabolite production, and nine strains were selected for study in PVC silos (codes in Figure 3). Strains associated with increased production of lactic acid (6; 39; 44 and 62); acetic acid (4; 9 and 60) and propionic acid (4; 9; 43; 44; 60; 62 and 65), and those that produced lower ethanol levels (6, 44 and 65) were chosen. Some of these strains also presented antimicrobial activity at *in vitro* tests against *Listeria monocytogenes* (ATCC 19117) (4; 6; 43 and 44); *Clostridium perfringens* (ATCC 3624) (6);

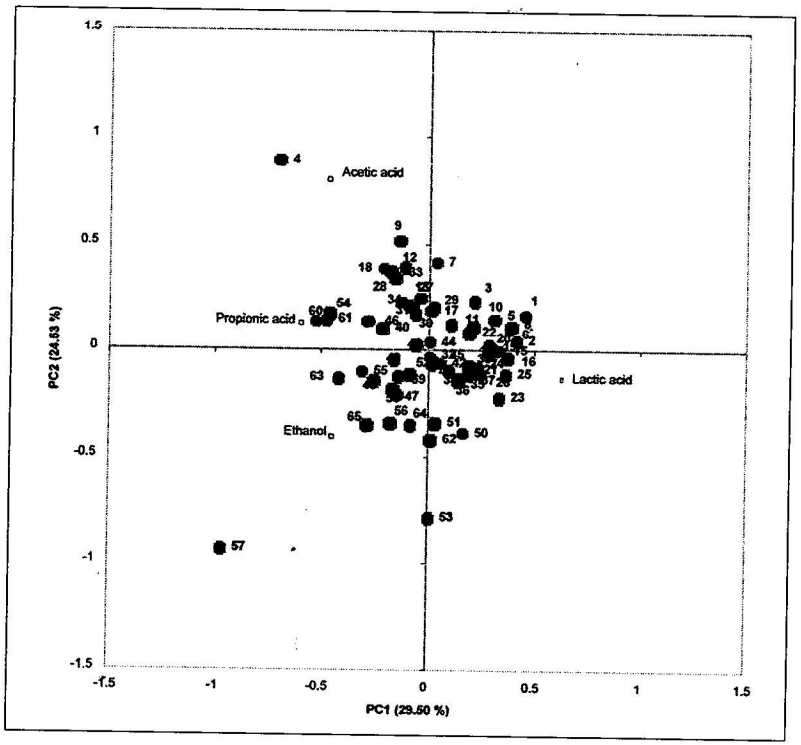

Figure 3. Principle components analysis of metabolites produced during fermentation of LAB strains in corn extract culture medium (Oliveira et al., 2011; data not published).

Escherichia coli (ATCC 11229) (39; 43; 44 and 62) and *Bacillus cereus* (ATCC 11778) (6).

Results for silage in experimental silos are presented in Table 4. Yeast, mold and total enterobacteria counts were not different across treatments at 60 days of fermentation. However, there were differences among LAB population and pH values. Two strains with high LAB counts also presented high pH values (strains 9 and 60). This is most likely related to their metabolism, since these strains were not classified as the best lactic acid producers but rather as good producers of acetic and propionic acid. On the other hand, strain 9 provided silage with the greatest aerobic stability, a lower maximum temperature (31.0°C) and a longer time period to reach this temperature (149.17 hours). The later is also related to metabolism. In other words, this strain is a good producer of acetic and propionic acids. These strains should be further studied for silage inoculation. Based on available data, they have high potential for use at corn ensiling.

The strain that provided the greatest stability in sugar cane silage (coded in Figure 1 and Table 3 as strain 55) was also tested in corn silage (coded as strain 65 in Table 4). In corn silage, this strain did not show the best results for aerobic stability, proving once again that selection should be specific.

Final considerations

Preliminary results of strain selection for both corn and sugarcane silage show that strains isolated from silage have great potential for use as inoculants. The wide variety of strains, their characteristics and performance in silage also became clear. In addition, it is important to highlight that use of a specific inoculant should not be generalized across cultures, since as discussed above, performance varies. Furthermore, when using different selection criteria, the most important one for the silage in question should be observed first eg. reduced DM loss in sugarcane silage. In this way, selection should be directed for the different cultures and based on various characteristics related to microbiological and chemical composition of silage.

Table 4. Populations of lactic acid bacteria (LAB), yeast, filamentous fungi (FF), enterobacteria and pH values of corn silage without inoculants and inoculated with different LAB strains.

Treatment*	LAB	Yeast	FF	Enterobacteria	pH	Aerobic Stability	
	Log UFC/g silage					Tmax[a] (°C)	Time[b](hours)
Control	7.25c	4.65	4.09	5.30	4.06b	37.83b	54.67c
Strain 4	7.96b	4.2	3.23	5.04	4.04b	37.83b	54.50c
Strain 6	7.06c	3.40	3.76	4.45	4.02b	39.00b	41.17d
Strain 9	8.72a	4.88	4.37	5.17	4.16a	31.00a	149.17a
Strain 59	7.36c	3.97	2.15	4:45	4.05b	37.17b	71.33b
Strain 43	7.59c	4.94	1.92	5.14	4.03b	38.67b	48.5d
Strain 44	6.87c	4.35	3.22	4.87	4.09b	38.83b	34.33d
Strain 60	8.34b	2.18	2.84	3.87	4.14a	38.67b	58.00c
Strain 62	7.38c	4.40	2.32	5.0	4.03b	39.83b	37.33d
Strain 65	8.12b	4.38	4.12	4.47	4.06b	37.83b	71.83b
P	0.000	0.2	0.33	0.53	0.0002	<0.001	<0.001
CV(%)	3.76	25.27	42.79	19.46	0.74	3.51	15.35

[a] Tmax.: maximum temperature observed during 10 days of air exposure.
[b] Time: time to reach maximum temperature.
Means followed by the same letter in a column do not differ statistically by the Scott-Knott test at a 5% significance level.
* Strain codes are same as those presented in Figure 1(PCA).

In conclusion, these are only preliminary results from a study that may be considered to still be in an initial phase, but one that should be continued and expanded. To do so, the study of the fermentation process of different cultures is of extreme importance. Tropical cultures should be emphasized since published data is lacking in this area.

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Improved efficiency of sugarcane ensiling for ruminant supplementation

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Introduction

Sugarcane is among the main Brazilian agricultural products. It is estimated that by the year 2012 the country will be producing around 685 million tons in nine million ha (Agrianual, 2007), destined to alcohol and sugar production. Also, the use of sugarcane as forage for dairy and beef cattle in Brazil is increasing, becoming popular among traditional users of corn and sorghum silages.

The main advantage of sugarcane as forage for cattle is its high productivity of biomass production (over 100 t/ha) which results

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