Diversity of prokaryotes associated with sugarcane silage treated with chemical and biological additives using PCR-DGGE

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Introduction The calcium oxide promotes a reduction in levels of fiber in sugar cane silage, which improves the nutritional quality (Santos et al., 2008). However silages treated with calcium oxide have a high final pH value, which might promote the growth of undesirable microorganisms. The association between calcium oxide and other additives that can induce rapidly decrease in sugar cane silage pH value could be interesting to reduce the fiber content without the risk of undesirable fermentation. In this sense, the use of microbial inoculants or weak organic acids may be an option to increase the speed and amplitude of the drop in pH of silage treated with calcium oxide. The PCR-DGGE (Electrophoresis in denaturing gradient gel) allows analyzing PCR products, according to their nucleotide sequences and size of products and has been widely used in studies of microbial communities in diverse environments. The objective of this study was to evaluate, using molecular techniques, the diversity of prokaryotes in sugar cane silage treated with selected *Lactobacillus* sp., calcium oxide, and propionic acid

Materials and Methods As experimental silos were used plastic buckets (20L). The additives tested were: calcium oxide (quicklime), propionic acid, both at a dosage of 1% based on fresh forage mass (FF) and a wild strain of *Lactobacillus* sp., (8.2 log cfu/kg forage) previously isolated from sugar cane silage (Ávila et al., 2009). Combinations of these additives were also evaluated and the silos were opened after 170 days of storage. To assess the diversity of prokaryotes were prepared samples of inoculant *Lactobacillus* sp. (UFLA SIL72) (A); of fresh sugar cane (B); of sugar cane silage without additives (control) (C); with microbial inoculant (D), with propionic acid (E), with calcium oxide (F), with the combination of the inoculant and propionic acid (G), with the combination of the inoculant and calcium oxide (H), with the combination of the propionic acid and calcium oxide (I) and with the combination of three additives (J). Total DNA was extracted from samples using the kit PSP Spin Stool DNA (Invitec ®) and for the amplification of the same the 518r and 338f primers of rDNA V3 region were used. Was used for PCR Master Mix kit (Quiagen) and 4 mL of DNA template. The amplicons were separated by DGGE (BioRad Universal Dcode Mutation Detection System, USA) in gel 8% polyacrylamide with denaturing gradient from 20 to 50%. Electrophoresis was performed in a constant voltage of 85 V for 16 h with a constant temperature of 60 ° C. The amplicons were excised, purified, re-amplified and sequenced.

Results and Discussion The results showed a heterogeneous profile of the samples analyzed. It was found eleven different bacteria species, and the respective bands excised: 1, 7, 17= *Lactobacillus hilgardii*; 2, 8 = *L. diolivorans*; 3, 10 = *L. parabuchneri*; 4= *L. acetotolerans*; 5, 6, 11, 20, 21= *L. kefiri*; 9= *L. brevis*; 10= *L.parabuchneri*; 12= *L. rhamnosus*; 13, 14, 18, 23= uncultivable microorganism; 15= *L. amylotrophicus*; 16, 22= *Proteus mirabilis*; 19= *L. parafarraginis*. The silage treated only with calcium oxide showed the highest number of amplicons, suggesting greater bacterial diversity in this silage. This treatment may have favored the growth of microorganisms due to the buffering of acids produced during fermentation

(Klosterman et al., 1960). The sample of fresh forage showed no amplicon, probably due to the limited number of copies of the 16S rRNA in the sample, might due to low number of cells. The bacteria (A) inoculated on silage, amplicons numbered as 1, 2, 7, 8 and 17, remained during whole fermentation, as observed in the samples with inoculant (D) and with association of inoculant and calcium oxide (H). In silage where both *Lactobacillus* sp and propionic acid were added (samples G and I) the amplicons were not observed. Probably the addition of propionic acid had a negative effect on the bacteria The presence of amplicons of non-cultivable microorganisms (13, 14, 18 and 23) showed the necessity of more detailed studies about the diversity of microorganisms associated with sugar cane silage.

Conclusion The PCR-DGGE technique was able to differentiate the bacterial profile of different samples of silage, therefore it is a good tool for monitoring bacteria species during the evaluation of inoculants in silage.

References

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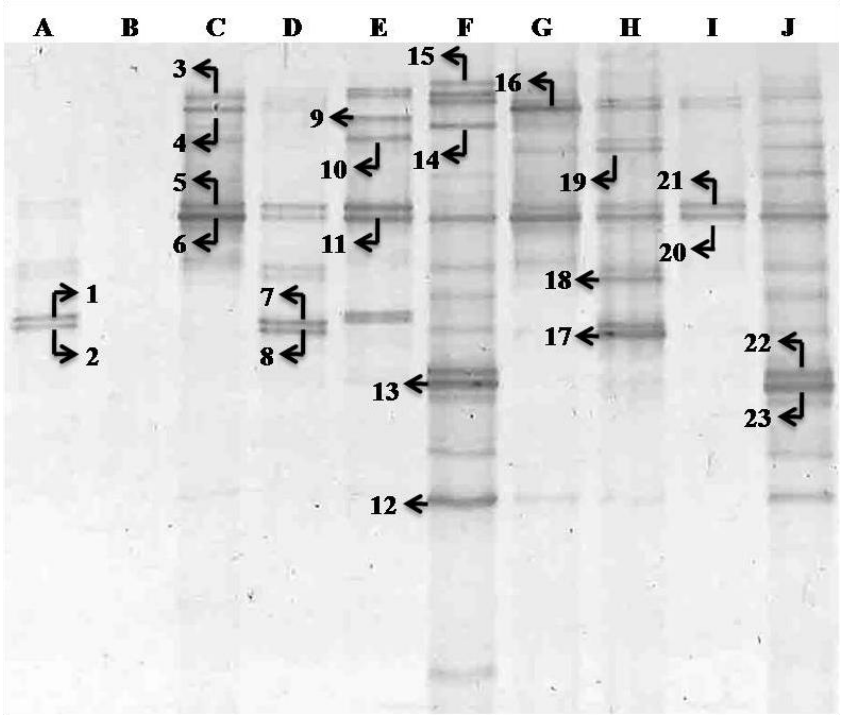

Figure 1 Bacterial communities associated with sugar cane silage treated with additives.