PCR-DGGE analysis of the dynamic diversity of lactic acid bacteria in alfalfa silage

B.Y. Zhang, Y.Y. Zang, Y.W. Zhang, C.C. Xu, F.Y. Yang
Grassland Institute, China Agricultural University, Beijing, China
Email: yfuyu@126.com

Introduction Most regions of China are affected by monsoons. Since the harvesting period for alfalfa often occurs in the rainy season, alfalfa crops are at a high risk of losses due to deterioration during the drying process. This leads to a lack of forage resources in the winter and early spring (Sheperd et al., 1995). The present study investigated the effects of different water content on the fermentation quality of alfalfa silage. A PCR-DGGE method was used to study the dynamic diversity of lactic acid fermenting bacteria in alfalfa silage and to track changes in the population structure of lactic acid bacteria strains. The study lays a solid foundation for the development of new inoculant products.

Materials and Methods Alfalfa (*Medicago sativa*) silage materials were collected from China Agricultural University and divided into three groups based on moisture content: low (60.7%), medium (71.2%), and high (80.3%) moisture content. They were ensiled for 0, 1, 5, 10, 20, 30, and 45 days and then the genomic DNA from silage microbes was extracted by the phenol-chloroform method. The target fragment was amplified by using the following primers: 357F (5′CCTACGGGAGGCAGCAG-3′,3′)-GC (5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGG) and 517R (5′-GTGCCAGC(A/C)GCCGCGG-3′). The dynamic fermentation diversity of the bacterial populations in the alfalfa silage was studied by PCR-DGGE (Han et al., 2009).

Results and Discussion Figure1 shows amplification bands that were located around 300bp, in agreement with a target fragment length of 280bp. The bands were sufficiently intense to be used for DGGE analysis, with the following results:

(1) A significant difference was found between the DNA bands among alfalfa silage samples ensiled at different durations. The numbers of bands revealed the diversity of the bacterial population in silage and the intensity of the bands indicated the relative numbers of bacteria.

(2) Almost all of the DNA samples, except for high moisture silage samples stored for 30 and 45 days, showed similar bands, which included No.1 (*Lactobacillus plantarum*), No.5 (*Lactobacillus farciminis*), No.7 (*Lactobacillus pentosus*), and No.10 (*Lactobacillus plantarum*). However, the bands varied in intensity, which indicated that the moisture content had a slight effect on the population sizes of these bacteria (Figure 2).

(3) Band No. 10 was consistently intense, suggesting that it came from a dominant bacterial population. Band No.4 showed a similar persistence, but its intensity was not as strong as Band 10 (Figure 2).

(4) The intensity of Band No. 1 increased during the later periods of fermentation, which may reflect the establishment of an improved environment for reproduction during storage. In contrast, other bands, such as Band No. 2, disappeared over time, perhaps due to the decrease in pH

occurring in the middle and late periods of silage fermentation, which may have led to declines in some aerobic bacterial populations (Figure.2)

Conclusion The 357F-GC and 517R primers were able to amplify genomic fragments from alfalfa silage bacteria and the DNA fragments were effectively separated using the DGGE method. The moisture content had little effect on the size or distribution of the bacterial population. Alfalfa silage contained great numbers of lactic acid bacteria, primarily *Lactobacillus* that showed as 7 bands, and small numbers of cocci.

References

Sheperd ACM, Maslanka, Quimn D, Kung JR. 1995. Additive containing bacteria and enzymes for alfalfa silage. J. Dairy Sci. 78: 555-572.

Han J., Yang K., Zhao Z. 2009. Corn and alfalfa silage fermentation system of flora diversity in PCR-DGGE analysis. Anhui Agri. University 36 (4):526-532.

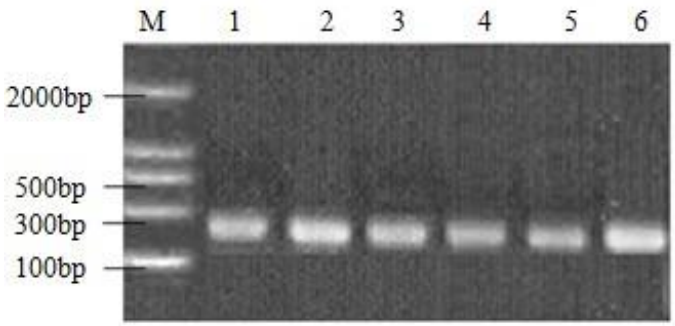

Figure 1. PCR results for microorganisms in alfalfa silage at low and high moisture content. M, 100bp marker; Lane 1-3, alfalfa silage at high, medium, low moisture, respectively. All samples were obtained after 1 day of ensilage; lane 4-6, alfalfa silage in high, medium, low moisture. All samples were obtained after 45 days of ensilage.

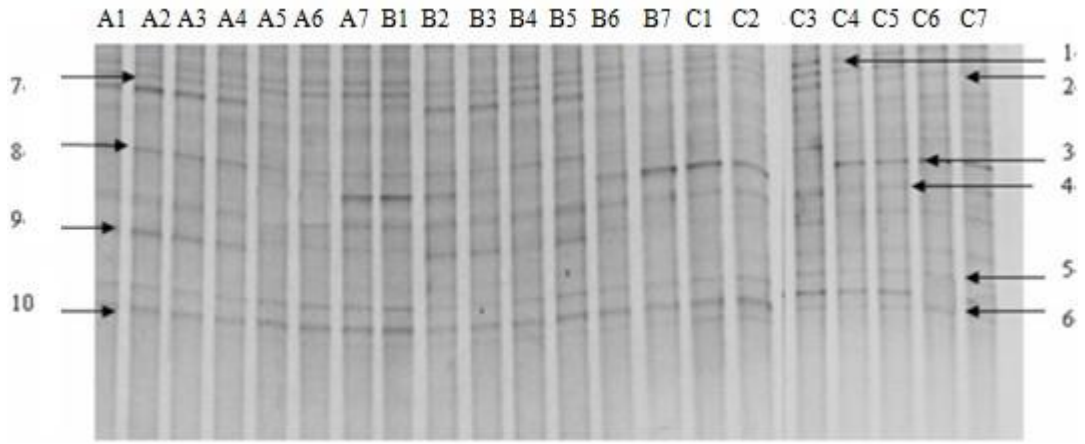

Figure 2. Electrophoretic DNA profiles of microorganisms in alfalfa silage. Lanes A1-A7: low moisture alfalfa silage after 0,1,5,10,20,30, and 45 days of ensilage; lanes B1-B7: medium moisture alfalfa silage after 0,1,5,10,20,30, and 45 days of ensilage; Lanes C1-C7: high moisture alfalfa silage after 0,1,5,10,20,30, and 45 days of ensilage.