Effect of lactic acid bacteria on silage quality of sweet sorghum (*Sorghum bicolor*)

Mala, A*; Fadlalla, B**; Mohamed, E***; Wang, S****; Shao, T*****

*Environmental, Natural Resource and Desertification Research Institute, National Center for Research, Ministry of High Education, Khartoum 11111, Sudan

**Sudan University of Science and Technology, College of Forestry &Range Sciences, Department of Range Science, Sudan.

***Key Laboratory of Yellow River Water Environment in Gansu province, Lanzhou Jiaotong University, Lanzhou 730070, China.

****Institute of Ensiling and Processing of Grass, Nanjing Agricultural University, Weigang 1, Nanjing 210095, China.

Keywords: Fermentation quality; Identification; Isolates; lactic acid bacteria, sweet sorghum.

Abstract

The purpose of this study was to identify lactic acid bacteria (LAB) from elephant grass silage throughout the fermentation process and investigate their effect on improving quality of sweet sorghum silage. The isolates were identified based on morphological, physiological, and biochemical features, as well as 16S rRNA profiling. A total of 120 lactic acid bacteria were isolates from elephant silage seven strains were purified and idntified three strains (*Pediococcus acidilactici* (AZZ1), *Lactobacillus plantarum subsp. plantarum* (AZZ4), *L. plantarum subsp. argentoratensis* (AZZ7) and one commercial bacteria *L. plantarum, ecosyl* MTD/1(CB)) were chosen as additives at 6 log colony forming units per gram of fresh sweet sorghum grass in laboratory silos (1000 g). Silos for each treatment were opened after 30, 60, and 90 days. All isolates were Gram-positive, catalase-negative, and grew properly in 65% sodium chloride. The strains AZZ1, AZZ2, and AZZ5 were classified as the *Pediococcus genus*, while AZZ3, AZZ4, AZZ6, and AZZ7 were *Lactobacillus genus*. Compared to the control, all the isolates enhanced the silage quality of sweet sorghum silage, evidenced by significantly ($P < 005$) decreasing pH, ammonia-nitrogen contents, undesirable microbe counts, and greater lactic acid (LA) contents. During ensiling, AZZ4 performed better among all inoculants, indicated by significantly ($P < 005$) lowered pH and ammonia-N contents and increased LA contents. In conclusion, strain AZZ4 is recommended as starter culture for tropical and subtropical grasses.

Introduction

In recent years, the demand for dairy products has increased in many developing countries as well as the tropical and subtropical regions of Asia and Africa. However, the production of silage for dairy farming has been hindered in these regions because of ensiling process that is highly dependent on local environmental conditions (Sifeeldein et al., 2019). To produce high-quality silage consistently in these regions, acid-tolerant, thermophilic lactic acid

bacteria (LAB) or homolactic acid fermented LAB must be identified and used as starter strains. (Sifeeldein et al., 2019). Because of the limitations of available technology, screening, selecting, and constructing starting cultures for silage production remains difficult, as is the classification of isolated strains. Closely related species, such as *Lactobacillus plantarum, Lactobacillus casei, Lactobacillus rhamnosus,* and *Lactobacillus pentosus*, which are the primary dominating strains in silage, are difficult or impossible to differentiate based on their phenotypes and genotypes (Duar et al., 2017).

In contrast, sorghum is one of the most suited plants for silage production and is becoming an increasingly significant feed crop in many regions of the world (Xie & Xu, 2019). Due to its high water soluble carbohydrates contents (WSC) and low buffer capacity, it is easy to ensile (Klevenhusen & Zebeli, 2021). It may also be an acceptable choice for silage production in marginal locations due to its high fodder output and drought tolerance. Inoculants have been proven to increase silage quality, as evidenced by lower pH and a greater number of lactic acid bacteria (LAB) (Guo et al., 2023).

Material and Methods

Sweet sorghum (*Sorghum bicolor)* grown at experimental field of Nanjing Agricultural University, Jiangsu, China. The grass was harvested at the mature stage.

Silage preparation

The chopped grasses were inoculated with three strains of LAB, *Pediococcus acidilactici* (AZZ1), *Lactobacillus plantarum subsp. plantarum* AZZ4, *L. plantarum subsp. argentoratensis* (AZZ7) and a commercial LAB *Lactobacillus Plantarum*, Ecosyl MTD/1 (CB) Ecosyl Product Inc. USA. LAB applied as additives at 6 colony forming units (cfu)/g calculated based on the fresh material weigh; Triplicate jars for each treatment were opened on days 30, 60, and 90 of ensiling.

Chemical analyses

The ammonia- N (NH_3-N) was determined according to the method of phenol-hypochlorite reaction. The pH of fresh grasses and silage were measured using a pH meter .Organic acids, including the lactic acid (LA), acetic acid (AA), propionic acid (PA) and butyric acid (BA) were analyzed by high-performance liquid chromatography according to the methods described by Mala et al.

Microbial population

A sub-sample (10 g) of wet silage from each sample was mixed with 90 mL of sterile saline solution (8.50g L^{-1}). Enumeration of LAB, aerobic bacteria, and yeast was performed using de Man, Rogosa, and Sharpe agar, nutritional agar, and potato dextrose agar, respectively. Finally, the total microbiological data were converted to log10 and presented on a fresh weight basis.

Results and Discussion

All isolates were identified as Gram-positive, catalase negative, rod-shaped bacteria. Compared to the control, all the isolates improved the silage quality of sweet sorghum silage, indicated by significantly ($P < 005$) lower pH and ammonia-nitrogen contents and undesirable microorganism counts, and higher lactic acid (LA) contents. During ensiling, AZZ4 performed better among all the inoculants, indicated by a significantly ($P < 005$) decreased pH and ammonia-N contents and a higher increase in LA contents.

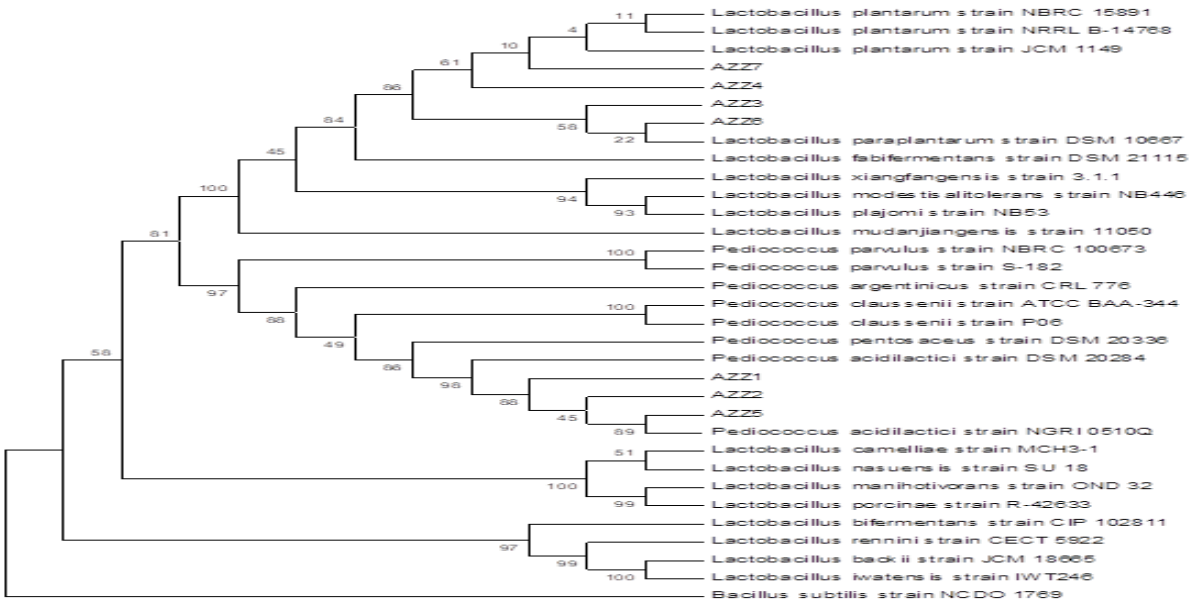

Figure 1 Phylogenetic tree of partial 16S rDNA sequences of isolated strains AZZ1, AZZ2, AZZ3, AZZ4, AZZ5, AZZ6 and AZZ7 isolate from elephant grass silage and sequences of identified bacteria in the nucleotide database of GenBank.

Effect of LAB on organic acids, pH and ammonia nitrogen of sweet sorghum silage

Effect of LAB on organic acids and ammonia nitrogen of sweet sorghum silage is shown in Fig 2. The addition of lactic acid bacteria isolates caused a higher level of LA, resulting in more decrease in pH and ammonia content than the control. The contents of acetic acid (AA) of all silages increased from 30 d to 60 days of ensiling, whereas the AA in the inoculated silages were lower (P<0.05) than the control. Propionic acid and butyric acid contents increased during ensiling and inoculated silage had lower PA and BA content than the control.

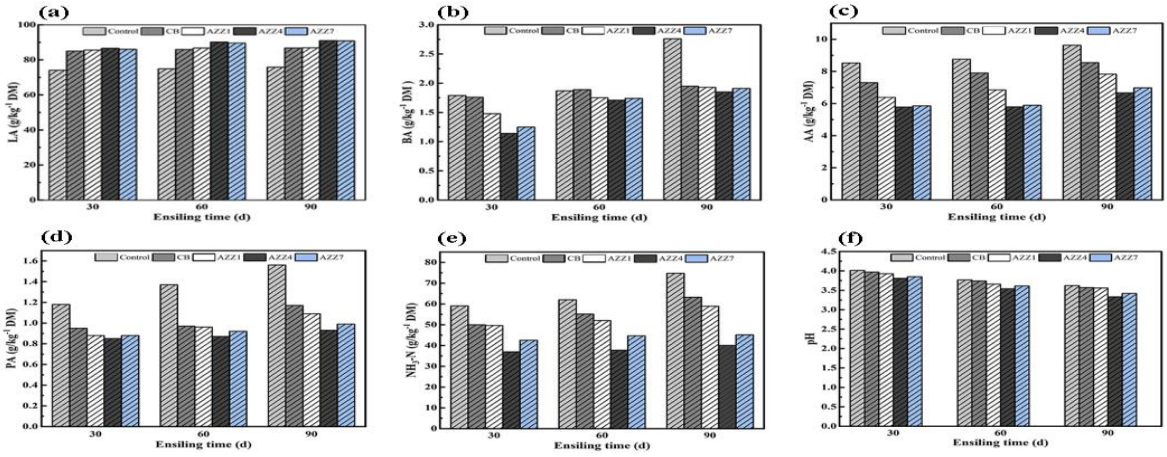

Figure 2. Effect of LAB on organic acids and ammonia nitrogen of sweet sorghum during fermentation period (a) LA: lactic acid, (b) AA: acetic acid, (c) PA: propionic acid, (d) BA: butyric acid. (e)NH_3-N: Ammonia nitrogen CB: Commercial bacteria.

Effect of lactic acid bacteria on microbiological compositions of sweet sorghum silage during ensiling

The effect of isolated strains on the microbiological compositions of the sweet sorghum silage after 30, 60 and 90d of ensiling shown in Figure 3. The population of microbial affected significantly ($P < 0.05$) by LAB addition

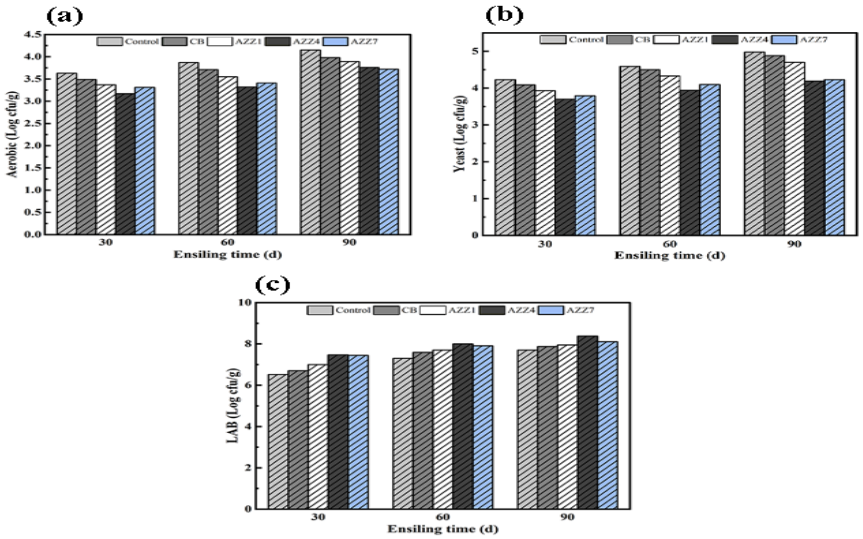

Figure 3. Effect of LAB on microbial composition of sweet sorghum silage during fermentation period, (a) LAB counts, (b) Aerobic bacteria counts, (c) Yeast counts of sweet sorghum silage. CB: Commercial bacteria, AZZ5: *Pediococcus acidilactici*, AZZ4: *Lactobacillus plantarum subsp. Plantarum*

Conclusions

In this experiment, the addition of AZZ1, AZZ4 and AZZ7 as inoculants significantly ($P < 005$) reduced the pH of the sweet sorghum silages and improved silage quality. Inoculants were efficient in improving fermentation quality, reducing NH_3-N as well as dry matter losses of sweet sorghum silage.

Acknowledgements

This work was supported by the project of cultivation, processing and utilization of forage in high temperature and humidity area (2017YFD0502106), National Natural Science Foundation of China (31672488) and Tibet Science and Technology Major Projects of Pratacultural Industry (2018ZDKJZC)

References

Duar, R. M., Lin, X. B., Zheng, J., Martino, M. E., Grenier, T., Pérez-Muñoz, M. E., Leulier, F., Gänzle, M., & Walter, J. (2017). Lifestyles in transition: evolution and natural history of the genus Lactobacillus. *FEMS Microbiology Reviews*, *41*(February), S27–S48. https://doi.org/10.1093/FEMSRE/FUX030

Guo, X., Xu, D., Li, F., Bai, J., & Su, R. (2023). Current approaches on the roles of lactic acid bacteria in crop silage. *Microbial Biotechnology*, *16*(1), 67–87. https://doi.org/10.1111/1751-7915.14184

Klevenhusen, F., & Zebeli, Q. (2021). A review on the potentials of using feeds rich in water-soluble carbohydrates to enhance rumen health and sustainability of dairy cattle production. *Journal of the Science of Food and Agriculture*, *101*(14), 5737–5746. https://doi.org/10.1002/jsfa.11358

Sifeeldein, A., Wang, S., Li, J., Dong, Z., Chen, L., Kaka, N. A., & Shao, T. (2019). Phylogenetic identification of lactic acid bacteria isolates and their effects on the fermentation quality of sweet sorghum (Sorghum bicolor) silage. *Journal of Applied Microbiology*, *126*(3), 718–729. https://doi.org/10.1111/jam.14123

Xie, Q., & Xu, Z. (2019). Sustainable Agriculture: From Sweet Sorghum Planting and Ensiling to Ruminant Feeding. *Molecular Plant*, *12*(5), 603–606. https://doi.org/10.1016/j.molp.2019.04.001