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Isolation and Identification of Lactic Acid Bacteria Strains and their Effects on the Fermentation Quality of Elephant Grass (*Cenchrus purpureus*) Silage

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Abstract

This study aims to isolate and identify lactic acid bacteria and examine their effects on the fermentation quality of elephant grass silage. The isolated strains were identified based on morphological, physiological and biochemical characteristics as well as 16S rRNA analysis. Three strains namely *Pediococcus acidilactic* (AZZ5), *Lactobacillus plantarum* subsp. *Plantarum* (AZZ4), *Lactobacillus plantarum* subsp. *Argentoratensis* (AZZ6) were isolated from elephant grass silage. Isolation of the microbes was done by serial dilution method. Three LAB and one commercial bacteria *Lactobacillus Plantarum*, *Ecosyl* MTD/1(CB)) were used as additives to fresh material of elephant grass. To follow the fermentation quality during ensiling, samples were taken on days 30, 60 and 90 of ensiling for chemical analysis. The strain AZZ5 was identified as *Pediococcus* genus while AZZ4 and AZZ6 were *Lactobacillus* genus. Compared to the control, all the isolates improved the silage quality of elephant grass silage. In conclusion, AZZ4 performed better among all inoculants.

Keywords: Elephant grass, Lactic acid bacteria, Isolates, Fermentation, *Lactobacillus plantarum*, *Pediococcus acidilactic*

1. Introduction

Elephant grass (*Cenchrus purpureus*) is a monocot C4 perennial grass in the *Poaceae* family, and it is among the highest yielding tropical grasses. Nevertheless, elephant grass is regarded as one of the most important tropical forages because of its high potential for biomass production, easy adaptation to diverse ecosystems and good acceptance by animals. A few homofermentative LAB commonly used in silage inoculants, including *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Enterococcus faecium* and *Pediococcus acidilactici* (Ennahar et al. 2003). According to a microbiological point of view to our knowledge, no information is accessible on the microbial ecology isolated from elephant grass silage, especially about the indigenous LAB and their effects on fermentation during ensiling. Currently, elephant grass has become more attractive due to its high yielding tropical grasses and good palatability for the animal, which can make it suitable for the LAB inhabit. For this reason, we chose elephant grass as research material for LAB isolation and selection.

This study set out to isolate, screen and identify lactic acid bacteria from elephant grass and their effect on fermentation quality of elephant silage during the fermentation process.

2. Material and Methods

2.1 Isolation and screening LAB

Ten grams of elephant grass were blended with 90 ml of sterilized saline solution (8.50 g L⁻¹NaCl). Each LAB colony was isolated and purified twice by streaking on MRS agar plates.

2.2 Extraction of lactic acid bacteria genomic DNA

Bacterial DNA was extracted according to the method of (Zoetendal et al. 1998) by a mini-bead beater. The genomic DNA concentration of each strain was determined by UV-VIS Spectrophotometer at 260 nm. The nucleotide sequences for the 16S rRNA gene were deposited to the GeneBank under accession numbers of AZZ4, AZZ5, and AZZ6 were KY584256, KY584255 and KY 584254 respectively.

2.3 Silage preparation

Strain AZZ4, AZZ5 and AZZ6 were chosen as additives at 6 log colony forming units (cfu)/g of fresh elephant grass. Experimental treatments included: control silage without LAB, elephant grass + AZZ5, elephant grass + AZZ4, elephant grass + AZZ6 and elephant grass +CB. Three silos from each treatment were opened after 30, 60 and 90 days of ensiling, respectively.

2.4 Chemical analysis

The WSC was measured by the anthrone method (Arthur 1977). NH₃-N was determined by the phenol-hypochlorite procedure (Kleinschmit et al., 2005). The organic acid and ethanol contents of the silage were analyzed by Agilent HPLC 1260.

3. Results

3.1 16S rRNA Gene Sequence Analysis

After blasting the 16S rRNA sequence, Strains AZZ4 and AZZ6 were clustered in the genus *Lactobacillus* with 81% similarity among their 16S rDNA gene sequences, on the other hand, strain AZZ5 was clustered in the genus *Pediococcus* with 99% similarity in their 16S rDNA gene sequences.

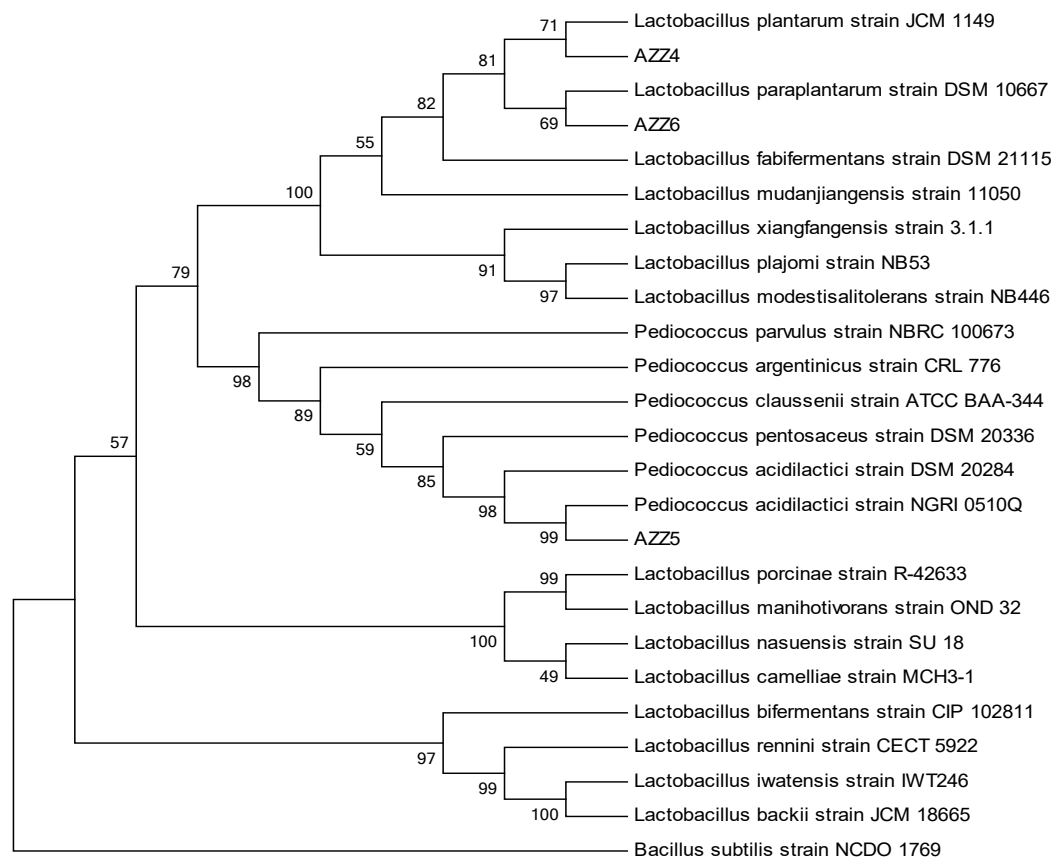


Fig 1. Polygenetic tree showing the relative positions of strains AZZ4, AZZ5, and AZZ6 isolate from elephant silage and related species, as inferred by the neighbour-joining method using complete 16S rRNA sequences.

3.2 Effect of LAB isolates on pH, WSC, NH3-N of elephant grass during ensiling

Effect of LAB on fermentation quality of elephant grass 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.

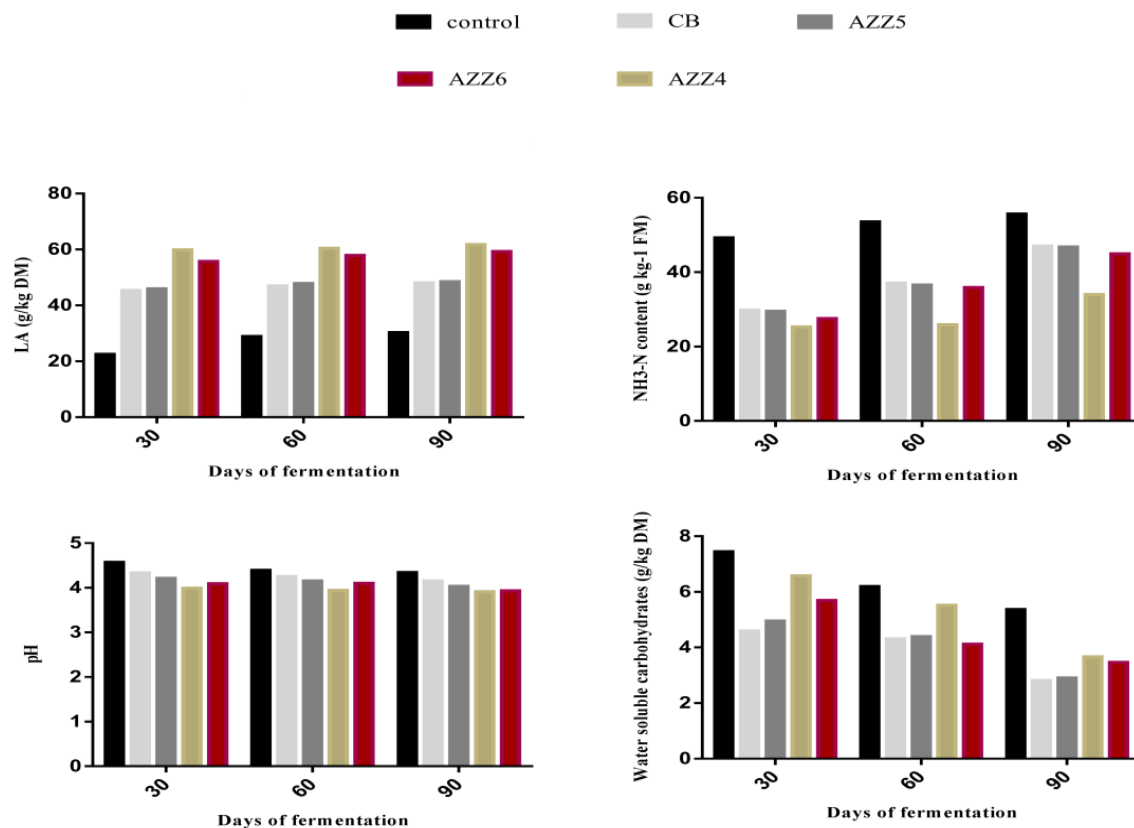


Fig2. Effect of LAB isolates on pH, LA, WSC, and NH3-N of elephant grass silage during ensiling

4. Discussion

Differentiation between isolates of known species using phenotypic methods is inconsistently successful, while the reported use of 16S rRNA sequence analysis is considered a good approach to identify LAB strains at both the genus and species level (Björkroth et al. 2002). However, some LAB species, for example, *L. plantarum* and *L. pentosushave* very similar 16S rRNA gene sequences, differing only by 2 bp (Hammes and Vogel 1995). Bacterial inoculants are added to forage at ensiling to stimulate lactic acid (LA) fermentation by accelerating the decrease in pH, thus improving silage preservation (McDonald et al. 2002). All inoculants improved the ensiling fermentation as apparent from a faster decrease in pH of elephant grass silage which is consistent with previous studies (Fellner et al. 2001).

5. Conclusion

Applying LAB isolates on elephant grass significantly influenced fermentation quality. The LA content increased by applying LAB isolates during ensiling. *L. plantarum subsp. plantarum* (AZZ4) had a better fermentation quality.

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