# INVOLVEMENT OF 4-PENTENOIC ACID IN CAUSING QUALITY DETERIORATION OF NETTLE SILAGE: STUDY OF ANTIBACTERIAL MECHANISM

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## INTRODUCTION

Ensiling legumes such as alfalfa presents challenges because of their high buffer capacity and protein content, as well as their low levels of water-soluble carbohydrates (WSCs), especially for nettle (Zhang *et al*., 2010). Recently, researchers showed that inoculation with *Pediococcus sp*. can improve the quality of legume silage by increasing lactic acid (LA) production and reducing pH levels (Kuppusamy *et al*., 2020). The relative abundance of spherical LAB, such as *Pediococcus spp.*, remains stable in nettle silage (Huang *et al*., 2023). However, various substances, including condensed tannins and flavonoids, can inhibit *Pediococcus sp.* activity during ensiling or under specific conditions (Bae *et al*., 2023). We investigated whether *Pediococcus sp.* can influence fermentation characteristics or overcome these challenges in nettle silage.

## MATERIAL AND METHODS

*Pediococcus sp*. strains were isolated from nettle silage under natural fermentation conditions. The nucleotide sequence data of the bacteria were deposited in the National Center for Biotechnology Information under accession number "OL455604" (accessed on November 19, 2021). Nettle (*Urtica cannabina*) was harvested on September 20, 2022, from the wild in Shawan County in the central mountains of Tianshan, Xinjiang, China (E 84°58′–86°24′; N 43°26′-45°20′). *P. pentosaceus* was evenly sprayed on each bag of the silage surface and completely mixed for storage. There were four fermentation period treatments (7, 15, 30, and 60 h), two inoculation treatments, and five replicates for each treatment, resulting in 40 bags of samples. Fresh silage samples (20 g) were used to analyze fermentation characteristics. The LA, acetic acid (AA), propionic acid (PA), pH, and ammonia (AN) levels were measured as described previously (Huang *et al*., 2023). Samples were selected after 7, 30, and 60 d of ensiling. Total DNA was extracted from each silage sample using a commercial DNA Kit (FastDNA® Spin Kit for Soil, MP Biomedicals, Irvine, CA, USA). The amplicons were purified and analyzed as previously described (Huang *et al*., 2023). For metabolite analysis, 50 g of fresh sample was collected on each natural fermentation day (after 7, 30, and 60 d of ensiling) of nettle silage, with five replicates for each silage. The minimum inhibitory concentrations of the substances against *P. pentosaceus* were determined using the broth dilution method. The characteristic data of the two silage groups were analyzed using Student's t-test. Data were analyzed using SPSS 22 Statistics (SPSS, Inc., Chicago, IL, USA). Differences between treatments were considered significant at $P < 0.05$. Bioinformatic analysis of the silage microbiota and metabolites was performed using the Majorbio Cloud platform (https://cloud.majorbio.com).

## RESULTS AND DISCUSSION

As shown in Table 1, during fermentation, The LA content was highest but the AA content was lowest in PP-treated plants compared with that in control plants after 7 d of ensiling ($p < 0.05$). The AN content was lower in the PP group than in the control group after 30 and 60 d of ensiling ($p < 0.05$). A ratio of LA-to-AA < 3 indicates heterolactic fermentation during ensiling, in which LAB such as *Lactobacillus buchneri* can convert WSC into LA, AA, and $CO_2$, resulting in a decrease in the DM. In the present study, inoculation with *P. pentosaceus* did not change the fermentation type or lead to a LA-to-AA ratio <3, but the content of AA decreased by 21.62% after 7 d of ensiling, indicating that *P. pentosaceus* can reduce DM loss during the early stage of ensiling fermentation. In this study, inoculation with *P. pentosaceus* decreased AN production during nettle ensiling. AN is correlated with undesirable bacteria (such as *Clostridium* and *Enterobacter*) and plant proteolytic enzyme activity, both of which are inhibited at a pH below (Bae *et al*., 2023; Ren *et al*., 2020; Ozogul *et al*., 2016). However, we showed that the pH was > 7 after inoculation, indicating that another factor was responsible for the reduction of AN during ensiling. Some species of *Pediococcus* can significantly decrease AN production (87.48% decline) in *Escherichia coli* (Ozogul *et al*., 2016). Thus, our results suggest that *P. pentosaceus* can inhibit undesirable bacterial activity during nettle ensiling.

*Pediococcus pentosaceus* was isolated from naturally fermented nettle silage, the correlation between *Pediococcus* and organic acids such as 4-pentenoic acid ($R^2 = -0.6323$, $p < 0.001$) and gluconic acid ($R^2 = -0.2512$, $p < 0.001$) was extremely negatively significant during nettle ensiling. The minimum inhibitory concentrations of 4-pentenoic acid and gluconic acid against *P. pentosaceus* were 16 and 32 mg/mL, respectively. We first observed that 4-pentenoic acid was present during the natural fermentation of nettle silage and that its content was almost stabilized during ensiling (decreased by 4.48% after 90 d of fermentation), inhibiting *P. pentosaceus* in vitro.

## CONCLUSION

Inoculation with *P. pentosaceus* decreased ammonia production (from 9.00% to 6.10% of total nitrogen) in nettle silage. However, the quality of nettle silage cannot be improved because of interference by 4-pentenoic acid, which exerts antibacterial effects.

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**Tab. 1:** The fermentation characteristics of nettle silage % dry matter

| Items | Ensiling days | Treatment | | SEM | P value |
|---|---|---|---|---|---|
| | | CK | PP | | |
| pH | 7 | 7.92 | 7.64 | 0.132 | < 0.001 |
| | 15 | 7.77 | 7.87 | 0.083 | 0.263 |
| | 30 | 8.35 | 8.37 | 0.058 | 0.059 |
| | 60 | 8.46 | 8.01 | 0.132 | < 0.001 |
| LA | 7 | 2.60 | 2.80 | 0.227 | 0.18 |
| | 15 | 3.27 | 3.58 | 0.459 | 0.273 |
| | 30 | ND | ND | – | – |
| | 60 | ND | ND | – | – |
| AA | 7 | 1.85 | 1.45 | 0.218 | 0.027 |
| | 15 | 1.92 | 2.05 | 0.360 | 0.238 |
| | 30 | ND | ND | – | – |
| | 60 | ND | ND | – | – |
| PA | 7 | 0.06 | 0.05 | 0.015 | 0.414 |
| | 15 | 0.09 | 0.09 | 0.032 | 0.097 |
| | 30 | ND | ND | – | – |
| | 60 | ND | ND | – | – |
| AN (%TN) | 7 | 1.06 | 1.48 | 0.25 | 0.167 |
| | 15 | 3.59 | 4.12 | 0.33 | 0.254 |
| | 30 | 6.71 | 5.05 | 0.55 | 0.039 |
| | 60 | 9.00 | 6.14 | 0.11 | < 0.001 |

CK: control; PP: inoculation with *P. pentosaceus*; LA: lactic acid; AA: acetic acid; PA: propionic acid; AN: ammonia nitrogen; TN: total nitrogen.

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