Effects of class IIa bacteriocin-producing *Lactobacillus* species on fermentation quality and aerobic stability of alfalfa silage

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Introduction Extensive research in recent years has revealed the potential of bacteriocin produced by lactic acid bacteria (LAB) as alternatives to feed antibiotics for improving the preservation of forages. Previous study has shown that inoculating forage with bacteriocin-producing bacteria was more effective than bacteriocin-free controls at inhibiting the growth of *L. monocytogenes* (Amado et al., 2012). As it is a newly found bacteriocin, little information is available on the application of class IIa bacteriocin-producing LAB in ensiled forages. Thus, the objective of this study was to examine effects of class IIa bacteriocin-producing LAB on fermentation quality, microbial counts, and aerobic stability of alfalfa silage.

Materials and Methods The chopped forage was ensiled with or without two strains of class IIa bacteriocin-producing lactic acid bacteria, *Lactobacillus delbrueckii* F17 (**F17**) and *Lactobacillus plantarum* (BNCC336943) (**LPB**), and a proven bacteriocin-free inoculant *Lactobacillus plantarum* MTD-1 (NCIMB40027) (**LPN**) in the laboratory silo, each at an application rate of 1×10^6 cfu/g. The mini-silos were then stored in an air-conditioned room (25 ± 2°C) and ensiled for 3, 7, 14, 30 and 60 d. Three replicates were prepared for each treatment at each ensiling duration.

Results and Discussion As shown in Table 1. On d 3 of ensiling, LPB-treated silage had the lowest pH but by d 7, LPN-treated silage had the lowest pH followed by LPB and the control silages. Yet after ensiling for 14, 30 and 60 d, F17, LPB and LPN had reduced ($P = 0.001$) silage pH compared to the control and the lowest pH was consistently in the LPB-treated silages. In addition, application of F17 and LPB decreased the number of yeasts and molds relative to control and LPN-treated silages. Large numbers of yeasts and molds ($> 10^5$ $\log_{10}$ cfu/g) adversely affect the preservation of silage and may lead to rapid spoilage when the silage is exposed to air (Gerlach et al., 2013). Compared to the control silage, inoculant-treated silages had greater aerobic stability (Figure 1), and the greatest aerobic stability occurring in LPB, followed by F17 and subsequently LPN.

Conclusions Inoculation with the class IIa bacteriocin-producing LAB strains, *Lactobacillus delbrueckii* F17 and *Lactobacillus plantarum* (BNCC 336943), at ensiling of alfalfa improved silage fermentation quality, reduced counts of molds and yeasts at early fermentation stage and improved aerobic stability compared with the *Lactobacillus plantarum* MTD-1, which does not

produce bacteriocin.

Table 1. Effects of additives and ensiling time on pH and microbial counts of alfalfa silage

Items	Treatment[1]	Ensiling time (d)[3]					Mean	SEM	Significance[2]		
		3	7	14	30	60			T	D	T×D
pH	C	5.88^{aA}	5.84^{bAB}	5.87^{aA}	5.73^{aB}	5.60^{aC}	5.79^{a}	0.023	0.001	<0.001	<0.001
	F17	5.76^{aB}	5.96^{aA}	5.72^{bBC}	5.63^{bC}	5.34^{cD}	5.68^{ab}				
	LPB	5.56^{bB}	5.78^{bA}	5.56^{cB}	5.47^{cB}	5.29^{dC}	5.53^{c}				
	LPN	5.72^{aA}	5.54^{cB}	5.73^{bA}	5.66^{bA}	5.48^{bB}	5.62^{bc}				
Yeasts, log_{10} cfu/g	C	8.05^{aA}	2.74^{aC}	3.66^{aB}	ND^{cD}	ND^{D}	2.88^{b}	0.027	< 0.001	< 0.001	< 0.001
	F17	6.57^{cA}	ND^{bC}	3.06^{bB}	2.40^{bC}	ND^{C}	2.41^{c}				
	LPB	7.36^{bA}	ND^{bC}	2.77^{bB}	2.70^{abB}	ND^{C}	2.57^{c}				
	LPN	7.77^{abA}	2.70^{aB}	< 2.00^{cC}	3.05^{aB}	ND^{D}	3.10^{a}				
Molds, log_{10} cfu/g	C	4.70^{bA}	ND^{bD}	2.13^{aC}	ND^{cD}	3.59^{aB}	2.08^{b}	0.021	< 0.001	< 0.001	< 0.001
	F17	ND^{cC}	ND^{bC}	2.09^{aB}	ND^{cC}	2.30^{bA}	0.88^{c}				
	LPB	ND^{cB}	ND^{bB}	< 2.00^{bA}	< 2.00^{aA}	ND^{cB}	0.73^{d}				
	LPN	5.59^{aA}	3.00^{aB}	< 2.00^{bC}	< 2.00^{bC}	ND^{cD}	2.43^{a}				

Means in the same column with different lowercase letters differed ($P < 0.05$) and means in the same row with different uppercase letters differed ($P < 0.05$).

[1]C, control, no additive; F17, *Lactobacillus delbrueckii* F17; LPB, *Lactobacillus plantarum* (BNCC 336943); LPN, *Lactobacillus plantarum* MTD-1 (NCIMB 40027).

[2]T, treatment; D, ensiling time; T × D, the interaction between treatment and ensiling time.

[3]< 2.00, below the detection limit; ND, not detected.

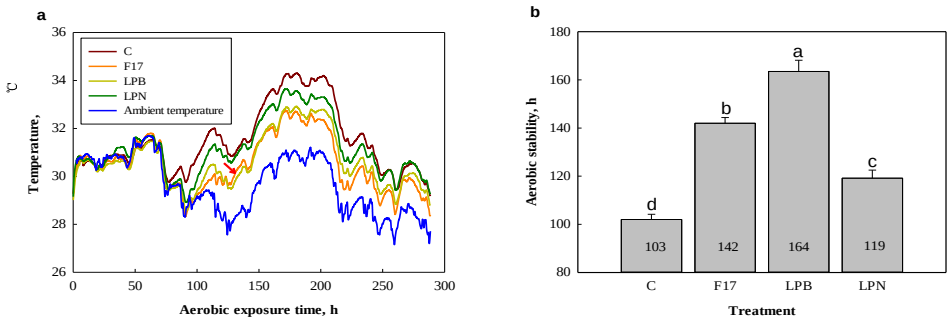

Figure 1. Effects of bacterial inoculants on aerobic stability of alfalfa silage. a, Temperature change during the aerobic phase; b, Aerobic stability of alfalfa silage treated without or with different inoculants.

References

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