The Effects of *Lactobacillus buchneri* 40788 and *Pediococcus pentosaceus* R1094 on the Fermentation of Corn Silage

D. H. Kleinschmit and L. Kung, Jr.¹

Department of Animal and Food Sciences University of Delaware, Newark 19716-2150

ABSTRACT

The effect of inoculating whole-plant corn at the time of harvest with *Lactobacillus buchneri* 40788 (4×10^5 cfu/g of fresh forage) combined with *Pediococcus pentosaceus* R1094 (1×10^5 cfu/g) on the fermentation and aerobic stability of corn silage (37% dry matter) through 361 d of ensiling was investigated. Dry matter recovery was similar between treatments throughout the study except at one early time point (14 d), when treated silage had a lower recovery than untreated silage. The concentration of lactic acid was unaffected by inoculation but inoculated silages had greater concentrations of 1,2-propanediol and acetic acid from 56 to 361 d of storage. In general, inoculation decreased the concentration of water-soluble carbohydrates but increased the concentration of ethanol. The numbers of yeasts was lower in inoculated silage at 42, 56, 70, and 282 d of ensiling. However, inoculation did not consistently improve the aerobic stability of silage, suggesting that microbes other than yeasts may have been responsible for aerobic instability in this study. Even after prolonged storage (361 d), silage treated with *L. buchneri* 40788 and *P. pentosaceus* R1094 had normal silage fermentation characteristics.

Key words: *Lactobacillus buchneri*, corn silage, aerobic stability

INTRODUCTION

Inoculating forages at harvest with *Lactobacillus buchneri* has improved the aerobic stability of the resulting silages (Muck, 1996; Weinberg et al., 1999; Taylor et al., 2002), most likely because this organism converts lactic acid to acetic acid under anaerobic conditions (Oude Elferink et al., 2001). A moderately high concentration of acetic acid can reduce the numbers of yeasts that cause spoilage when silage is exposed to air (Woolford, 1975). In some previous studies, the accumulation of acetic acid from the metabolism of *L. buchneri*

was not immediate and occurred after several weeks of fermentation (Ranjit and Kung, 2000; Taylor and Kung, 2002). Oude Elferink et al. (2001) reported that the conversion of lactic acid to acetic acid by *L. buchneri* occurred more readily at a low (<4) than high (>5) pH, but this does not fully explain the delayed response in most corn silages because a low pH is usually reached quickly in this crop.

In the majority of studies with *L. buchneri*, it usually has been the sole organism used for inoculation, and evaluation has often been conducted only after moderately short lengths of ensiling (60 to 120 d; Ranjit and Kung, 2000; Ranjit et al., 2002; Kleinschmit et al., 2005). However, on many farms, silages may remain stored for longer time periods. For example, silage fed in the summer of the following year would have been in storage for 8 to 9 mo. In addition, silage may be stored for even longer periods of time during robust growing seasons. Recently, *L. buchneri* has been marketed in combination with homolactic acid bacteria, which are commonly added to forages to increase lactic acid production, rapidly drop pH, and improve DM recovery (Kung et al., 2003), but there are no long term studies on the effects of this combination of bacteria on silage fermentation. Thus, the objectives of this study were to determine the effects of an inoculant containing both *L. buchneri* 40788 and *Pediococcus pentosaceus* R1094 on fermentation end-products, DM recovery, and aerobic stability of corn silage after various lengths of storage.

MATERIALS AND METHODS

Whole-plant corn, Pioneer 33Y18 (Pioneer Hi-Bred International, Des Moines, IA), was harvested at one-half milk line and 37.4% DM using a New Holland FP230 (New Holland, PA) pull-type harvester equipped with a mechanical processor. Roller clearance was set at 2 mm with a theoretical length of cut of 19 mm. Chopped forage was divided into two 300-kg piles. One pile was treated with 800 mL of deionized water, and the remaining pile was treated with an inoculant containing *L. buchneri* 40788 (final application rate of 400,000 cfu/g of fresh forage) and *P. pentosaceus* R1094 (final application rate of 100,000 cfu/g) that was dis-

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¹Corresponding author: lksilage@udel.edu

solved in 800 mL of deionized water. Prior to the experiment, the inoculant was plated on Rogosa SL agar (Difco-248020; Becton Dickinson, Sparks, MD), and based on the measured concentration of lactic acid bacteria, an appropriate amount was used to achieve the desired application rate. Samples of each pile of treated forage were collected prior to ensiling and frozen at -20°C for later analysis. Approximately 11 kg of fresh forage was packed in 20-L laboratory silos for each treatment to achieve a packing density of approximately 200 kg of DM/ m^3 . Silos were sealed with solid lids with O-ring seals. Triplicate silos for each treatment were allowed to ensile for 14, 28, 42, 56, 70, 282, and 361 d at ambient temperature (15 to 35°C) in a closed barn.

The DM content of forages and silages were determined by drying duplicate samples from each silo in a 60°C forced-air oven for 48 h. Dried samples of fresh forage and silages were ground with a UDY Cyclone sample mill (UDY Corp., Fort Collins, CO) through a 1-mm screen. Samples were analyzed for NDF by using sulfite and amylase (Van Soest et al., 1991) and for ADF (Robertson and Van Soest, 1981) using an Ankom²⁰⁰ fiber analyzer (Ankom Technology, Fairport, NY). Crude protein was determined by total combustion of the sample (Leco CNS 2000 Analyzer; Leco, St. Joseph, MI) and by multiplying total N by 6.25.

A water extract was prepared for each sample by adding 25 g of fresh forage or silage to 225 mL of 25% Ringer's solution (Oxoid BR52; Unipath, Basingstoke, UK) and homogenizing the mixture for 1 min. The pH of the water extract was measured and a portion of it was filtered through Whatman 54 filter paper (Whatman, Clifton, NJ). Nine milliliters of extract was acidified with 50 μL of 50% H_2SO_4 and frozen prior to analysis for ammonia-N (Weatherburn, 1967) and water-soluble carbohydrates (WSC; Nelson, 1944). Frozen water extracts were thawed twice and centrifuged at $10,000 \times g$ for 10 min and analyzed for concentrations of acetic, propionic, and butyric acids; 1,2-propanediol; and ethanol by HPLC (Muck and Dickerson, 1998). Freshly prepared water extracts were also filtered through a double layer of cheesecloth and enumerated for yeasts and molds by pour plating in malt extract agar (Oxoid CM59) that had been acidified with 85% lactic acid at a rate of 0.5% (vol/vol). Plates were incubated in a 32°C oven for 2 d and counted for numbers of yeasts.

Dry matter recovery for each silo was calculated based on the initial weight and DM content of the forage placed in the silos and the amount of silage DM remaining after ensiling. The aerobic stability of each silo was determined by loosely adding 3 kg of a representative sample of silage back to its respective silo and exposing it to air at 22 to 25°C . A thermocouple wire

Table 1. The chemical composition of freshly chopped whole-plant corn after treatment and prior to ensiling¹

Treatment	U	T	SE
DM, %	37.6	37.2	0.6
pH	5.75	5.73	0.02
NDF, %	45.9	50.3	2.2
ADF, %	29.2	29.5	1.7
CP, %	10.5	10.5	0.3
Ammonia-N, %	0.050	0.049	0.003
WSC, %	7.10	7.21	0.60

¹U = Untreated corn silage; T = corn silage treated with *Pediococcus pentosaceus* R1094 (1×10^5 cfu/g of fresh forage) and *Lactobacillus buchneri* 40788 (4×10^5 cfu/g of fresh forage); WSC = water-soluble carbohydrates.

was placed in the geometric center of the forage mass. The wire was attached to a data logger (model number CR10X; Campbell Scientific, Inc., Logan, UT) that recorded the temperature every 10 min and averaged these values every 2 h. Each silo was covered with a double layer of sterile cheesecloth to avoid contamination and drying of the forage, but allowing air to infiltrate the forage mass. Aerobic stability was defined as the number of hours silage remained stable before a 2°C rise in temperature above the ambient.

Microbial data were converted to $\log_{10}$ and reported on a wet weight basis, and chemical data are presented on a DM basis. The experiment was a randomized block design with days of ensiling as blocks; data were analyzed by using the GLM procedure of SAS (SAS Institute, 1998). Because of some missing data, mean separation was performed using least squares means. Significance was declared at $P < 0.05$ and trends were noted at $P < 0.10$.

RESULTS AND DISCUSSION

The chemical composition of freshly chopped forage was similar to that from past studies (Ranjit et al., 2002) and was not different between treatments (Table 1). The concentrations of CP, ADF, and NDF after fermentation were not affected by inoculation at any time point (data not shown). In a previous study, Kleinschmit et al. (2005) also reported that inoculation with *L. buchneri* did not affect these components. The DM content, pH, concentration of ammonia-N, and percent DM recovery of the silages after ensiling are reported in Table 2. The pH and ammonia-N of the silages were similar between treatments at all sampling times, with the exception that they were lower in inoculated silages at 361 d. The DM recovery of silages was also similar between treatments after all lengths of storage, with the exception that it was greater in untreated silage (96.8%) compared with treated silage (91.5%) at d 14. The concentrations of lactic acid (Figure 1) were

Table 2. The pH, DM, and $\text{NH}_3\text{-N}$ composition and DM recovery of corn silage after 14 through 361 d of ensiling¹

Item	14 d		28 d		42 d		56 d		70 d		282 d		361 d		SE	
	U	T	U	T	U	T	U	T	U	T	U	T	U	T		
DM, %	36.0 ^a	34.2 ^b	34.6	33.9	35.7	36.0	35.7	36.6 ^a	35.5 ^b	36.4	35.8	37.0	36.1	35.2	35.8	0.6
pH	3.71	3.71	3.69	3.73	3.71	3.73	3.71	3.64	3.70	3.73	3.73	3.60	3.67	3.89 ^a	3.77 ^b	0.03
NH ₃ -N, %	0.088	0.093	0.091	0.092	0.099	0.104	0.099	0.105	0.104	0.104	0.107	0.081	0.088	0.161 ^a	0.130 ^b	0.007
DMR, %	96.8 ^a	91.5 ^b	93.1	90.6	95.4	93.6	95.4	97.7	94.7	96.5	95.6	97.5	96.2	93.2	94.9	1.3

^{a,b}Means in rows within similar days of ensiling with unlike superscripts differ ($P < 0.05$).¹U = Untreated corn silage; T = corn silage treated with *Pedococcus pentosaceus* R1094 (1×10^5 cfu/g of fresh forage) and *Lactobacillus buchneri* 40788 (4×10^5 cfu/g of fresh forage); $\text{NH}_3\text{-N}$ = ammonia-N (DM basis); DMR = dry matter recovery. **Figure 1.** The concentration of lactic acid (DM basis) in untreated corn silage (open bars) or corn silage treated (solid bars) with *Lactobacillus buchneri* 40788 (4×10^5 cfu/g of fresh forage) and *Pedococcus pentosaceus* R1094 (1×10^5 cfu/g of fresh forage). SE = 0.31.

between 5 and 6% of the DM and were similar between treatments, regardless of the length of storage. The results for DM recovery, pH, and lactic acid are in contrast to the general expectations of silages treated with only *L. buchneri*. In a meta-analysis of published studies, Kleinschmit and Kung (2006) reported that inoculation with $>100,000$ cfu of *L. buchneri* (alone) per gram of forage resulted in corn silage with a higher pH, a lower concentration of lactic acid, and a slightly greater loss of DM when compared with untreated corn silage. However, Filya (2003) reported that treating sorghum and corn silages with *L. buchneri* combined with *L. plantarum* (a homolactic acid bacterium) resulted in intermediate effects compared with treatment of those crops with *L. buchneri* alone. Thus, our results may also reflect this fact.

Concentrations of acetic acid were somewhat low in all treatments (Figure 2) relative to what is normally observed for corn silage (Ranjit and Kung, 2000; Ranjit et al., 2002). This resulted in calculated ratios of lactic acid to acetic acid that were greater than 3 (data not shown), which has been used as an indicator of a good homolactic acid fermentation. Greater concentrations of acetic acid, a hallmark of silages treated with *L. buchneri*, were observed in inoculated than in untreated silages from 56 d of ensiling onward. The increase in concentration in acetic acid because of inoculation with *L. buchneri* has occurred at different times. Filya (2003) observed this effect within 2 d of ensiling in low-DM corn and sorghum silages, whereas Driehuis et al. (1999) reported that it took 20 d to observe the effect in corn silage. In high moisture corn, Taylor and

Figure 2. The concentration of acetic acid (DM basis) in untreated corn silage (open bars) or corn silage treated (solid bars) with *Lactobacillus buchneri* 40788 (4×10^5 cfu/g of fresh forage) and *Pediococcus pentosaceus* R1094 (1×10^5 cfu/g of fresh forage). ^{a,b}Bars within a day with unlike letters differ ($P < 0.05$). SE = 0.08.

Figure 3. The concentration of 1,2-propanediol in untreated corn silage (open bars) or corn silage treated (solid bars) with *Lactobacillus buchneri* 40788 (4×10^5 cfu/g of fresh forage) and *Pediococcus pentosaceus* R1094 (1×10^5 cfu/g of fresh forage). ^{a,b}Bars within a day with unlike letters differ ($P < 0.08$). SE = 0.07.

Kung (2002) did not report a consistent increase in the concentration of acetic acid from inoculation with *L. buchneri* until after 49 d of ensiling. In the current study, the difference in concentrations of acetic acid between untreated and inoculated corn silage increased with length of storage through 281 d, but these increases were of a moderate nature even after prolonged storage. After 361 d, the difference, although still significant, diminished primarily because acetic acid increased in untreated silage. Untreated silage had undetectable concentrations of 1,2-propanediol until d 361 (<0.2%). The concentration of 1,2-propanediol in silage treated with *L. buchneri* was not detected until 42 d of sampling (Figure 3) and generally increased with prolonged storage thereafter, reaching a peak concentration of more than 0.6% at 361 d. The lack of propionic acid detected in any inoculated silage, coupled with increasing concentrations of 1,2-propanediol, suggested that the epiphytic population of microbes did not have the capacity to further convert 1,2-propanediol to propionic acid.

The concentration of WSC (Figure 4) in inoculated silage was lower than levels found in untreated silage throughout the ensiling period, with the exception of d 42 and 361. Generally, inoculating silages with *L. buchneri* has resulted in lower concentrations of WSC in silages, but the differences in the concentrations of WSC at the early sampling points observed in the current study were probably due to an increased use of sugars by the addition of *P. pentosaceus* and not to *L. buchneri*. Treated silages had greater concentrations of

ethanol (Figure 5) than untreated silages throughout the study, except at 282 d, when the concentrations were similar between treatments. This finding was unexpected because we would have predicted lower concentrations of ethanol as a result of a more dominant homolactic acid fermentation from the addition of *P.*

Figure 4. The concentration of water-soluble carbohydrates (WSC, DM basis) in untreated corn silage (open bars) or corn silage treated (solid bars) with *Lactobacillus buchneri* 40788 (4×10^5 cfu/g of fresh forage) and *Pediococcus pentosaceus* R1094 (1×10^5 cfu/g of fresh forage). ^{a,b}Bars within a day with unlike letters differ ($P < 0.05$). SE = 0.13.

Figure 5. The concentration of ethanol (DM basis) in untreated corn silage (open bars) or corn silage treated (solid bars) with *Lactobacillus buchneri* 40788 (4×10^5 cfu/g of fresh forage) and *Pediococcus pentosaceus* R1094 (1×10^5 cfu/g of fresh forage). ^{a,b}Bars within a day with unlike letters differ ($P < 0.05$). SE = 0.10.

pentosaceus and from a reduction in the numbers of yeasts. The greater concentration of ethanol in treated silages may have been caused by the fermentation of lactic acid to acetic acid by *L. buchneri* (Oude Elferink et al., 2001).

The numbers of yeasts detected in silage (Figure 6) were similar between treatments until 42 d of ensiling,

Figure 6. The number of yeasts in untreated corn silage (open bars) or corn silage treated (solid bars) with *Lactobacillus buchneri* 40788 (4×10^5 cfu/g of fresh forage) and *Pediococcus pentosaceus* R1094 (1×10^5 cfu/g of fresh forage). ^{a,b}Bars within a day with unlike letters differ ($P < 0.05$). ^{c,d}Bars within a day with unlike letters differ ($P < 0.10$). SE = 0.64.

Figure 7. The aerobic stability in untreated corn silage (open bars) or corn silage treated (solid bars) with *Lactobacillus buchneri* 40788 (4×10^5 cfu/g of fresh forage) and *Pediococcus pentosaceus* R1094 (1×10^5 cfu/g of fresh forage) during ensiling. ^{a,b}Bars within a day with unlike letters differ ($P < 0.05$). ^{c,d}Bars within a day with unlike letters differ ($P < 0.10$). SE = 19.

when inoculated silage contained more than 100-fold fewer yeasts than did untreated silage. This finding generally coincided with the greater concentrations of acetic acid in inoculated silage beginning at d 56. Numbers of yeasts declined at a faster rate and continued to be lower in treated vs. untreated silage through 70 d of storage, and tended ($P < 0.10$) to be lower at 282 d of storage. Driehuis et al. (1999) also reported that the numbers of yeasts declined in silages treated with *L. buchneri* with increasing time of storage. Although there were generally fewer yeasts in treated silages, aerobic stability was not consistently improved by inoculation (Figure 7). Treated silage tended ($P < 0.10$) to be more stable at 14 and 56 d of storage than untreated silage, and was more stable ($P < 0.05$) at d 361. Although inoculation with traditional homolactic acid bacteria has sometimes led to the worsening of aerobic stability (Weinberg et al., 1993), it is doubtful that the general lack of improvement in aerobic stability in inoculated silage in our study was caused by their inclusion in the current study because the numbers of yeasts decreased steadily in inoculated silage as the length of storage progressed. Driehuis et al. (2001) and Filya (2003) reported that inoculation with *L. buchneri* alone or in combination with a homofermentative lactic acid bacteria equally improved the aerobic stability of grass silage and corn silage, respectively. A more plausible explanation for the lack of consistent improvement in aerobic stability with inoculation is the fact that other organisms, such as acetic acid bacteria, may have initiated spoilage in the current study. These microbes have the

capacity to oxidize ethanol to acetic acid and further degrade lactic and acetic acids to carbon dioxide and water, thereby causing spoilage in silage (Spoelstra et al., 1988).

CONCLUSIONS

Treating whole-plant corn with *L. buchneri* 40788 and *P. pentosaceus* R1094 resulted in increased concentrations of acetic acid and 1,2-propanediol from 56 through 361 d of storage when compared with untreated corn silage. However, the numbers of yeasts in inoculated silage were lower than in untreated silage before the increase in acetic acid occurred, indicating that it may not have been the only inhibitor of yeasts in treated silage. Lack of a consistent improvement in aerobic stability despite the fact that inoculated silage had more acetic acid and fewer yeasts indicated that other organisms, such as acetic acid bacteria, may have initiated aerobic instability in the current study. The concentrations of most fermentation end-products (with the exception of 1,2-propanediol) remained within a normal range even after prolonged storage in inoculated silage.

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