Effect of inoculation of *Acetobacter pasteurianus* at ensiling and after silo opening on fermentation products, aerobic stability, and bacterial community of whole crop corn silage

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Introduction Acetic acid bacteria are obligate aerobes and obtain energy by oxidizing ethanol to acetic acid or oxidizing lactic acid to acetic acid and carbon dioxide. In a survey of bacterial community of bunker-made corn silage, we isolated *Acetobacter pasteurianus* as dominant bacteria grown in Man, Rogosa, Sharpe agar plates. The silage was not spoiled at the time of sampling, although acetic acid bacteria are known to initiate deterioration in the presence of air. To examine how *A. pasterianus* was involved in fermentation and aerobic spoilage, inoculation experiments were made using the isolate.

Materials and Methods Whole crop corn was harvested at the half milk-line stage on 17 September 2010. Finely chopped crop (300 g) was packed in plastic pouches, and stored at ambient temperature with and without *A. pasteurianus* (10^5 cfu/g) inoculation. Triplicate silages were opened at 2 months after ensiling, and fermentation products and bacterial community were determined at silo opening and after 7-day aerobic deterioration test. Untreated silage was also opened after storage for 6 months, and then added with and without *A. pasteurianus* (10^6 cfu/g) prior to the 7-day aerobic deterioration test. Bacterial community was determined by denaturing gradient gel electrophoresis, and species identification was made based on the partial 16S rRNA gene sequence (DGGE). Presence of *A. pasteurianus* was also evaluated by amplifying PQQ-dependent alcohol dehydrogenase gene.

Results and Discussion Inoculation of *A. pasteurianus* at ensiling increased silage pH, while not affecting lactic acid and acetic acid contents (Table 1). Aerobic stability was improved by *A. pasteurianus* addition; heating was delayed for 2 days and lower pH than untreated control was observed after aerobic deterioration test. Bacterial community was similar between control and *A. pasteurianus*-inoculated silage, and *Lactobacillus plantarum* and *Lactococcus lactis* were found as 2 representative bacteria (Figure 1a). Improved aerobic stability was reflected in bacterial community; although *Bacillus pumilus* appeared upon spoilage in control silage, marked changes were not seen before and after aerobic deterioration test in *A. pasteurianus*-inoculated silage. The band indicative of *A. pasteurianus*, however, was not detectable in any silage, suggesting that *A. pasteurianus* did not survive the ensiling process and was not directly involved in improved aerobic stability.

When *A. pasteurianus* was inoculated to untreated silage after silo opening, spoilage occurred without heating and the lactic acid and acetic acid contents decreased. Spoilage was not accelerated by addition of *A. pasteurianus*, although the band indicative of *A. pasteurianus* became distinctive during the process of aerobic deterioration (Figure 1b). Presence of *A. pasteurianus* in DGGE gel agreed well with detection of PQQ-dependent alcohol dehydrogenase gene in the silage (Figure 2).

Conclusion These findings suggest that *A. pasteurianus* can be a follow-up or an opportunistic bacterium after silo opening, and its contribution to initiating aerobic deterioration may be small compared with that previously reported.

Table 1 Chemical and microbial composition at silo opening and after 7-day aerobic deterioration test of whole crop corn silage inoculated with and without *Acetobacter pasteurianus* (AP) at ensiling

Item	At silo opening			After aerobic stability test		
	Control	AP	SE	Control	AP	SE
Dry matter (%)	41.8	43.3	0.24	45.9	51.1	0.23
pH	3.68 b	3.74 a	0.01	5.98 x	4.94 y	0.09
Lactic acid (%DM)	3.96	4.77	0.66	0.61	1.03	0.12
Acetic acid (%DM)	0.72	0.76	0.08	0.09 x	0.01 y	0.01
Ethanol (%DM)	1.54	1.49	0.18	0.00 y	0.03 x	0.01
Lactic acid bacteria (log cfu/g)	6.10	6.20	0.07	8.89	8.71	0.12
Yeasts (log cfu/g)	5.90	6.30	0.13	9.05	9.08	0.07

Values within the same row with unlike following letter are different (P<0.05).

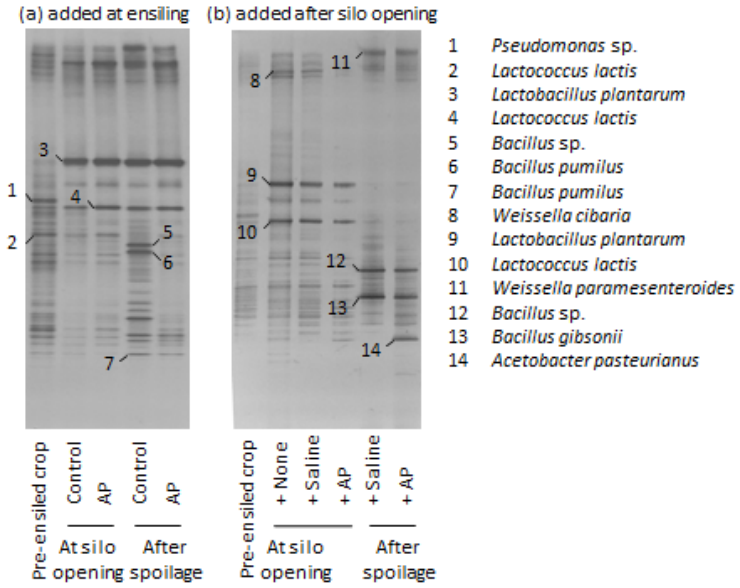

Figure 1 Bacterial community at silo opening and after 7-day aerobic deterioration test of whole crop corn silage inoculated with and without *Acetobacter pasteurianus*. Inoculation was made at ensiling (a) or after silo opening of untreated silage (b).

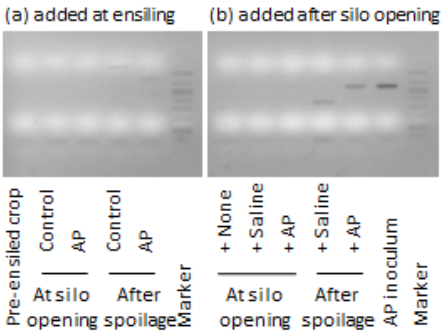

Figure 2 Determination of PQQ-dependent alcohol dehydrogenase gene at silo opening and after 7-day aerobic deterioration test of whole crop corn silage inoculated with and without *Acetobacter pasteurianus*. Inoculation was made at ensiling (a) or after silo opening of untreated silage (b).