Bacterial community of direct-cut and wilted guinea grass silage stored with and without molasses

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Introduction Tropical grass ensiling may result in large amounts of acetic acid rather than lactic acid. Even if lactic acid is prevalent in the beginning, the level of lactic acid may decrease and that of acetic acid will increase when ensiling is prolonged. Information is still insufficient on the bacterial community of tropical grass ensiling, and microorganisms associated with acetic acid fermentation have not been identified. In this study, guinea grass was ensiled immediately after harvest or after sun light wilting to prepare high-moisture tropical grass silage. Molasses was added to obtain various fermentation patterns, and changes in the bacterial community were assessed by denaturing gradient gel electrophoresis.

Materials and Methods The first growth of guinea grass was harvested in the late heading stage, on August 4th, 2009. The grass was chopped using a forage cutter at a theoretical length of 13 mm immediately after harvest or after field wilting for about 6 h. The pre-ensiled materials were added with and without molasses (1.0% fresh basis), and stored in a plastic pouch at ambient temperature for 14, 28, and 56 days. Microbial counts, fermentation products, and bacterial community were determined.

Results and Discussion Intensive acetic acid fermentation occurred in untreated direct-cut silage; the lactic acid content was only 0.39 %DM and the acetic acid content was as high as 5.41 %DM at day 14 (Table 1). The acetic acid level remained high during ensiling, and the silage pH remained above 6.0 even at day 56. Marked increases were seen in the butyric acid, ethanol and NH_3-N content due to prolonged ensiling. Addition of molasses increased the lactic acid content and decreased the propionic acid, ethanol and NH_3-N content. Butyric acid was not detectable in direct-cut silage to which molasses had been added, whereas about 5.0 %DM of acetic acid was detected from day 14 onwards.

Wilting enhanced the lactic acid production even without adding molasses (Table 2). Lactic acid was a major product in untreated wilted silage. The lactic acid content decreased and the acetic acid content increased when ensiling was prolonged, and the levels of these acids were comparable at day 56. In addition, the ethanol content decreased and the NH_3-N content increased due to prolonged ensiling. Both the lactic and acetic acid contents were high in wilted silage to which molasses had been added; hence, the lactic to acetic acid ratio was unaffected by molasses addition. Similar to untreated wilted silage, prolonged ensiling decreased the lactic acid content, ethanol content and lactic to acetic acid ratio, and increased the NH_3-N content.

Although bands for various enterobacteria, such as *Pantoea agglomerans*, *Acinetobacter* sp., and *Pantoea ananatis*, were found in direct-cut and wilted materials, bands for these species were not detectable after ensiling (Figure 1). Distinctive bands indicative of *Morganella morganii*, *Lactobacillus plantarum*, *Enterococcus* sp., *Pantoea* sp., *Lactococcus lactis*, and *Enterococcus faecium* were found at day 14 in untreated direct-cut silage. At days 28 and 56, prominent bands for *Morganella morganii* and *Clostridium botulinum* were also observed. In direct-cut silage to which molasses had been added, bands indicative of *Pediococcus pentosaceus* were clearly seen, whereas those indicative of *E. faecium* were not observed. The DGGE patterns for untreated wilted silage were similar to those for direct-cut silage to which molasses had been added;

however, bands indicative of *M. morganii* and *Pantoea* sp. were faint in untreated wilted silage. A distinctive band for *Lactococcus garvieae* was seen in wilted silage to which molasses had been added.

Table 1 Microbial counts and fermentation products of direct-cut guinea grass silage

	Direct-cut + untreated			SE	Direct-cut + molasses			SE	ANOVA		
	14 d	28 d	56 d		14 d	28 d	56 d		S	M	SxM
Dry matter (%)	13.9	13.2	13.2	0.60	15.4	15.3	15.3	0.36	NS	**	NS
pH	6.28	6.67	6.06	0.13	5.06	4.89	4.75	0.06	**	**	*
Lactic acid (%DM)	0.39	0.12	0.01	0.07	3.24	3.72	3.09	0.23	NS	**	NS
Acetic acid (%DM)	5.41	5.14	5.64	0.50	4.86	5.37	5.32	0.41	NS	NS	NS
Propionic acid (%DM)	0.13	0.29	0.34	0.06	0.00	0.14	0.13	0.01	**	**	NS
Butyric acid (%DM)	0.13	1.20	1.65	0.39	0.00	0.00	0.00	0.00	*	**	*
Ethanol (%DM)	1.01	2.90	4.19	0.46	0.64	0.83	0.90	0.16	**	**	**
NH_3-N (%DM)	0.34	0.47	0.55	0.05	0.27	0.30	0.25	0.03	*	**	*

S, M, and SxM indicate effect of storage period, molasses addition, and their interaction, respectively.

Table 2 Microbial counts and fermentation products of wilted guinea grass silage

	Wilted + untreated			SE	Wilted + molasses			SE	ANOVA		
	14 d	28 d	56 d		14 d	28 d	56 d		S	M	SxM
Dry matter (%)	23.5	22.5	25.0	1.27	20.5	21.1	21.4	0.61	NS	**	NS
pH	4.90	4.99	4.87	0.05	4.44	4.61	4.67	0.07	NS	**	NS
Lactic acid (%DM)	3.62	3.13	2.84	0.25	5.30	4.75	4.11	0.17	**	**	NS
Acetic acid (%DM)	1.19	2.67	3.08	0.20	2.10	3.19	3.91	0.28	**	**	NS
Propionic acid (%DM)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	---	---	---
Butyric acid (%DM)	0.00	0.00	0.06	0.02	0.00	0.00	0.00	0.00	NS	NS	NS
Ethanol (%DM)	0.58	0.40	0.29	0.07	0.64	0.33	0.33	0.07	**	NS	NS
NH_3-N (%DM)	0.10	0.16	0.17	0.02	0.12	0.16	0.21	0.03	**	NS	NS

S, M, and SxM indicate effect of storage period, molasses addition, and their interaction, respectively.

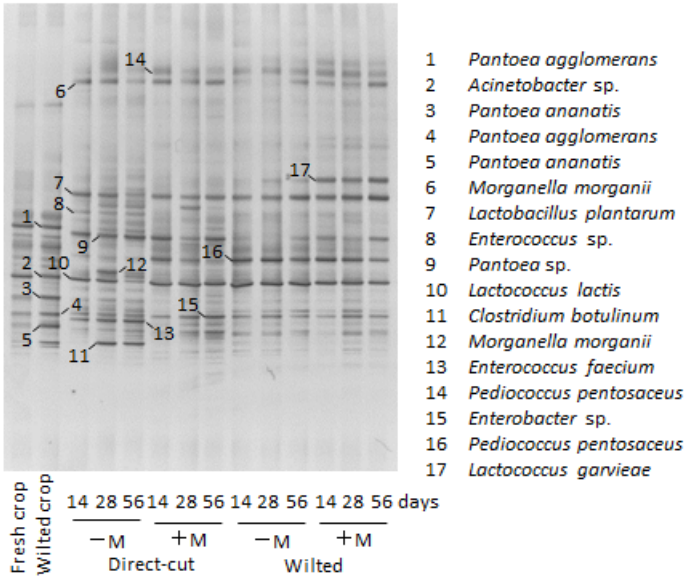

Figure 1 Bacterial community of direct-cut and wilted guinea grass silage stored with and without molasses (M) for 14, 28, and 56 days.