Ensiling of soybean curd residue and wet brewers grains with or without other feeds as a total mixed ration

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Ensiling of Soybean Curd Residue and Wet Brewers Grains With or Without Other Feeds as a Total Mixed Ration

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ABSTRACT

Wet brewers grains and soybean curd residue were stored in laboratory-scale silos without (BG and SC silages, respectively) or with other ingredients as total mixed rations (BGT and SCT silages, respectively). Silages were opened after 14 and 56 d, and microbial counts, fermentation products, and aerobic stability were determined. Denaturing gradient gel electrophoresis was carried out to examine bacterial communities, and several bacteria that appeared to be involved in fermentation were identified. Lactic acid content was greater in SCT than in BGT silage, but lower in SC than in BG silage. Ethanol content was greater in BG than in SC regardless of silage type. Aerobic deterioration occurred promptly in ensiling materials (nonensiled by-products and total mixed ration mixtures) and in silages stored alone; however, SCT and BGT silages resisted deterioration and no heating was found for more than 5.5 d regardless of storage period. Silages were stable even with high yeast populations at silo opening, whereas prolonged ensiling decreased yeast counts in the 2 total mixed ration silages. The denaturing gradient gel electrophoresis profiles appeared similar between SCT and BGT silages but not between SC and BG silages. *Weissella* spp. and *Lactobacillus brevis* were common in aerobically stable SCT and BGT silages, and *Lactobacillus buchneri* was detected only in BGT silage. Both *L. brevis* and *L. buchneri* were found in silage but not in ensiling materials. Several other lactic acid bacteria were also identified in SCT and BGT silages, but did not appear to be related to fermentation and aerobic stability.

Key words: by-product, denaturing gradient gel electrophoresis, silage, total mixed ration

INTRODUCTION

Because of economic and environmental concerns, the utilization of food by-products is expected to increase and to become more efficient. The Japanese government legislated a Food Recycling Act in 2001, and has promoted the "3Rs" (i.e., reduce, reuse, and recycle) of organic resources generated by the food industries. Many food by-products are high in moisture content and are thus often stored by ensiling to avoid the energy costs of drying. Although some wet by-products can be ensiled without any additives (Katayama, 2001; Osaka, 2001), much cost would be wasted on transporting water rather than nutrients. Therefore, a practice in Japan is to mix wet by-products with dry feeds to prepare low-moisture TMR silage (Abe, 2000; Imai, 2001). Wet brewers grains (**BG**) and soybean curd residue (**SC**) are often used as the main ingredients, and various feedstuffs such as grass hay, legume hay, cracked corn, soybean meal, and wheat bran are added in lower proportions (Abe, 2000). More than 10 ingredients are usually formulated to create a TMR silage, which is stored in thick polyethylene bags after vacuum packing. The DM, CP, and total digestible nutrient contents are 50 to 60%, 16 to 18% of DM, and 72 to 74% of DM, respectively, and silages are commercialized in the form of transportable (300 to 400 kg) bag silos (Nishino, 2006). Because silage manufacturers do not have enough space available to store a large numbers of bags, a short ensiling period is preferred.

Our previous study (Nishino et al., 2003a) showed that although deterioration occurred promptly (within 2 d after silo opening) when BG was ensiled alone, no heating was shown for 7 d when BG was stored as a TMR silage. We isolated *Lactobacillus buchneri* from stable TMR silage as a predominant lactic acid bacterium (**LAB**), and then found that the 1,2-propanediol-producing LAB can suppress aerobic deterioration of corn and grass silages (Nishino et al., 2003b, 2004; Nishino and Touno, 2005). Although the reason why *L. buchneri* predominated during fermentation is unknown, our findings suggest that *L. buchneri* may account for the stability of BG-containing TMR silage. Interestingly, deterioration was suppressed even with yeast counts >10^6 cfu/g at silo opening (Nishino et al., 2004; Nishino and Hattori, 2007; Wang and Nishino, 2008), despite the fact that silage with a high yeast

population ($>10^5$ cfu/g) is prone to deteriorate on exposure to air (McDonald et al., 1991). Yeast numbers decreased to below detectable levels when ensiling was prolonged, indicating that the stability of TMR silage can be further improved by a lack of undesirable microorganisms.

Previously, we examined the properties of TMR silage by using only BG as a wet by-product (Nishino et al., 2004; Nishino and Hattori, 2007; Wang and Nishino, 2008), although about 0.7 times more SC than BG is produced annually in Japan (Abe, 2000). Wet BG is a residue obtained after extracting wort from mashed malted grains, and SC is a pulp obtained after straining soy milk. The DM and CP contents are 75 to 80% and 26 to 27% of DM, respectively, in both by-products (Abe, 2000; Imai, 2001). Farmers and silage manufacturers have recognized a similar stability in SC-containing TMR silage; however, no experiments have been done to investigate whether *L. buchneri* would predominate during fermentation and produce 1,2-propanediol. It has been shown that SC is more difficult to ensile alone than BG, because aerobic bacteria may grow fast and spoilage would occur soon after disposal (Niwa, 2001).

The aim of present experiments was to evaluate the fermentation and aerobic stability of TMR silage prepared with BG and SC as the main ingredients. Microorganisms involved in fermentation and aerobic stability were examined by PCR-denaturing gradient gel electrophoresis (**PCR-DGGE**), and several bacteria were identified by 16S rDNA sequence.

MATERIALS AND METHODS

Ensiling

Two experiments (**Exp.**) were performed (Table 1). In Exp. 1, only TMR silage was prepared with SC or BG as a main ingredient (**SCT** and **BGT** silages). In Exp. 2, silages were prepared with SC and BG alone (SC and BG silages) or with other feeds (SCT and BGT silages). The TMR mixtures were formulated with wet by-products (SC or BG) at 50% on a fresh weight basis, and with alfalfa hay, sudangrass hay, cracked corn, wheat bran, beet pulp, soybean meal, and molasses at lower proportions. Targeted DM and total digestible nutrients were 55 and 74% of DM, respectively. The TMR were stored in vacuum-packed plastic pouches. Fresh by-products or TMR mixtures (300 g) were put into a pouch, and an airtight silo was prepared by using a vacuum sealer. Silages were made in triplicate and stored at ambient temperature (Exp. 1) or at 25°C (Exp. 2) for 14 and 56 d, respectively.

Aerobic Stability Test

After silage was completely opened, half the contents (150 g) were put into polyethylene bottles (500 mL) without compaction. The top of the bottle was kept uncovered and exposed to air for 7 d in a room maintained at 25°C. A conventional thermometer was placed in the center of the silage, and the temperature was recorded at 12-h intervals. Aerobic deterioration was judged to have started when the temperature reached 2°C above the surrounding temperature.

Chemical and Microbial Analyses

The DM contents were determined by freeze-drying. Silage pH, lactic acid, short-chain fatty acids, and alcohols were determined on water extracts (Nishino et al., 2004). Analysis of volatile fermentation products was performed by GC (GC-14A, Shimadzu, Kyoto, Japan) fitted with a glass capillary column (15 m × 0.53 mm) coated with polyethylene glycol terephthalic acid modified (TC-FFAP, GL Sciences, Tokyo, Japan). The temperature of column oven was programmed at 80°C for the first 2 min and thereafter increased to 200°C at a rate of 10°C/min. The numbers of LAB and yeasts were counted using de Man, Rogosa, and Sharpe agar, and potato dextrose agar, respectively. The pH of potato dextrose agar was adjusted to 3.5 by adding sterilized lactic acid solution after autoclaving. The serially diluted plates of LAB and yeasts were incubated at 30°C for 3 d.

DGGE

A frozen sample was thawed and added with 40 times the volume of sterilized PBS. Extraction was made by vigorous shaking for 10 min at ambient temperature, and microbial pellets were obtained by centrifugation at 8,000 × *g* for 15 min. Bacterial DNA was purified using a commercial kit (DNeasy Tissue Kit, Qiagen, Germantown, MD) according to the manufacturer's recommendation.

Polymerase chain reaction was carried out to amplify a variable region (V3 region) of the bacterial 16S rDNA with the GC-clamp forward primer GC357f (5′-CGCC CGCCGCGCGCGGCGGGCGGGGCGGGGGCACGG GGGGCCTACGGGAGGCAGCAG-3′) and the reverse primer 517r (5′-ATTACCGCGGCTGCTGG-3′). Amplification was done in a standard reaction mixture containing 25 m*M* Tris (hydroxymethyl) methyl aminopropane sulfonic acid (pH 9.3), 50 m*M* KCl, 2.0 m*M* MgCl$_2$, 0.2 m*M* dNTP, 2.5 U of *Taq* polymerase, 1.0 m*M* each primer, and the DNA template. The PCR was run with an initial denaturation at 95°C for 10 min, and 30 cycles of denaturation at 93°C for 30 s, annealing

Table 1. Ingredients and composition of wet by-products and their TMR mixtures

| | Treatment[1] | | | | | |
| | Experiment 1 | | Experiment 2 | | | |
Item	SCT	BGT	SC	BG	SCT	BGT
Ingredient (%)						
Soybean curd residue	50		100		50	
Wet brewers grains		50		100		50
Alfalfa hay	5	5			5	5
Sudangrass hay	5	5			5	5
Cracked corn	10	10			10	10
Wheat bran	10	10			10	10
Soybean meal	2	2			2	2
Dried beet pulp	10	10			10	10
Molasses	8	8			8	8
Composition						
DM (%)	55.4	58.9	23.3	25.3	55.6	57.5
CP (% of DM)	15.3	14.6	24.9	27.0	14.6	13.4
WSC[2] (% of DM)	6.37	6.94	3.36	4.74	6.69	8.34
pH	5.14	5.19	6.68	4.22	5.58	5.19
Lactic acid bacteria (log cfu/g)	8.65	6.30	<2.00	6.91	3.94	6.36
Yeasts (log cfu/g)	3.69	3.45	<2.00	3.79	3.26	<2.00

[1]SC = soybean curd residue; BG = brewers grains; SCT = TMR containing SC; BGT = TMR containing BG.

[2]Water-soluble carbohydrates.

at 65°C (first 10 cycles), 60°C (second 10 cycles), or 55°C (last 10 cycles) for 30 s, and extension at 72°C for 1 min, with a final extension at 72°C for 5 min (Pedro et al., 2001). The reaction was conducted in a PCR thermal cycler (Dice, Takara Bio Inc., Shiga, Japan).

The GC-clamp PCR products were separated according to their sequences with a DCode Universal Mutation System (Bio-Rad Ltd., Tokyo, Japan). The samples were applied directly onto 10% (wt/vol) polyacrylamide gels in a running buffer containing 20 mM Tris-acetate and 0.5 mM EDTA-2Na (pH 8.5). The gels were prepared with a denaturing gradient from 25 to 50% of urea and formamide. Electrophoresis was conducted at a constant voltage of 150 V for 12 h at 60°C. After electrophoresis, gels were stained with SYBR Green (Cambrex Bio Science Inc., Rockland, ME) and photographed under UV illumination.

Sequence Analyses of DNA Bands

Bands of interest were excised from DGGE gels and the pieces were soaked in 10 μL of sterilized water to allow diffusion of DNA. Extracted DNA was amplified by PCR using 357f (without GC-clamp) and 517r primers, and then PCR products were purified using a commercial cleanup kit (GeneClean Kit, Qbiogene, Carlsbad, CA). The sequencing reaction was carried out using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA), and DNA base sequences were analyzed by using an ABI PRISM 310 sequencer (Applied Biosystems). Searches in the Gen-

Bank database with the BLAST program were performed to determine the closest relatives of partial 16S rDNA sequences. Percentage identities ranged from 86 to 100%.

Statistical Analyses

In Exp. 1, data were subjected to ANOVA and differences between means were assessed by Student's *t*-test. In Exp. 2, a 2-way ANOVA was applied with by-product source and silage type as the main factors. These statistical analyses were performed by using JMP IN software (ver. 5.1.2, SAS Institute, Tokyo, Japan).

RESULTS

The DM contents of fresh BG and SC were as low as 25% (Table 1). Although the wet by-products were obtained soon after their disposal from factories, they contained a small amount of lactic acid, and the pH of BG product was reduced to 4.2. The LAB and yeasts counts were 10^7 and 10^4 cfu/g, respectively, in BG material, whereas their counts were below detectable levels in SC product. The DM contents were more than 55% in all TMR materials. In all TMR mixtures, the pH was greater than 5.0, and LAB and yeasts counts were 10^6 and 10^3 to 10^4 cfu/g, respectively.

Lactic acid was the main fermentation product in both SCT and BGT silages in Exp. 1 (Table 2). Lactic acid content was greater in SCT than BGT silage regardless of storage period, and the content increased

Table 2. Chemical and microbial composition of TMR silages containing soybean curd residue and brewers grains (experiment 1)

Item	Treatment[1]			
	SCT	BGT	SEM	*t*-test
Ensiling for 14 d				
pH	4.06	4.29	0.01	**
Lactic acid (%)	1.90	1.15	0.06	**
Acetic acid (%)	1.17	1.04	0.07	NS
Propionic acid (%)	0.01	0.02	0.00	**
Butyric acid (%)	0.01	0.01	0.00	NS
Undissociated acids[2] (%)	1.71	1.10	0.06	**
Ethanol (%)	0.07	0.88	0.04	**
1,2-Propanediol (%)	0.00	0.00	—	—
Lactic acid bacteria (log cfu/g)	8.68	8.28	0.03	**
Yeasts (log cfu/g)	2.57	6.10	0.49	**
Ensiling for 56 d				
pH	3.97	4.05	0.84	**
Lactic acid (%)	2.53	1.56	0.13	**
Acetic acid (%)	1.08	0.72	0.13	NS
Propionic acid (%)	0.00	0.01	0.00	NS
Butyric acid (%)	0.00	0.00	—	—
Undissociated acids[2] (%)	2.05	1.23	0.17	*
Ethanol (%)	0.03	0.73	0.13	*
1,2-Propanediol (%)	0.01	0.12	0.01	**
Lactic acid bacteria (log cfu/g)	7.22	7.30	0.28	NS
Yeasts (log cfu/g)	<2.00	2.47	—	—

[1]SCT = TMR containing soybean curd residue; BGT = TMR containing brewers grains.

[2]Sum of undissociated lactic acid and VFA.

*$P < 0.05$; **$P < 0.01$; NS = $P > 0.05$.

with prolonged ensiling. Ethanol content was greater in BGT than in SCT silage, and the production in 56-d BGT silage was comparable to acetic acid. 1,2-Propanediol was found in both 56-d silages, but only at trace levels (0.01%) in SCT silage. About 10^8 and 10^7 cfu/g of LAB were present in 14- and 56-d silages, respectively, regardless of by-product type. Although yeast counts exceeded 10^6 cfu/g in 14-d BGT silage, the numbers decreased to a nearly undetectable level (10^2 cfu/g) when ensiling was prolonged for 56 d. Yeast counts were less than 10^3 cfu/g in 14-d SCT silage, and yeasts were undetectable in 56-d silage.

When BG was ensiled alone, lactic acid was produced rapidly, reducing pH to below 3.5 after 14 d (Table 3). Lactic acid production was less in SC silage, and pH remained at 4.4 after 14 d of storage. Prolonged ensiling increased acetic acid content, and pH increased to 4.6 in 56-d SC silage. Ethanol production was greater in BG than in SC silage. Fermentation of TMR silage in Exp. 2 was similar to that in Exp. 1; lactic acid content was greater in SCT than in BGT silage, but the opposite result was shown for ethanol content. Greater ethanol content in 14-d BGT silage was associated with a higher yeast count. Prolonged ensiling decreased yeast counts, more so in SCT than in BGT silage. An interesting observation was that lactic acid production was greater in BG when ensiled alone, whereas production was

greater in SC when ensiled as a TMR. 1,2-Propanediol content was low in Exp. 2, and the maximum was 0.03% in 56-d BGT silage.

Aerobic deterioration occurred within 2 d in materials and in silages stored alone (Figure 1). One exception was 56-d SC silage, in which heating was detected after 4 d in the presence of air. The TMR silages resisted deterioration for more than 5.5 d regardless of by-product type and storage period. Ensiling for 56 d enhanced stability, and 14-d SCT and BGT silages appeared relatively stable.

In Exp. 2, *Weissella* spp. and *Lactobacillus brevis* were common in SCT and BGT silages (Figure 2). *Weissella* spp. were also found in SCT material, whereas a corresponding DNA band was not found in BGT material. *Streptococcus* spp. were detected in SCT material and appeared to survive ensiling for 56 d. In contrast, *Lactobacillus fermentum* in SCT material did not exist after 14 d of storage, and *Lactobacillus plantarum* in BGT material also disappeared during fermentation. *Lactobacillus brevis* was not present in either SCT or BGT material, but was detected after 14 d of storage in both TMR silages. *Lactobacillus buchneri* was also detected when BGT was ensiled, but the bacterium was not found in TMR material or in SCT silages.

Weissella spp. and *L. brevis* were also common in SCT and BGT silages in Exp. 2. *Lactobacillus brevis* was not detected in any of the materials, and was detected only in TMR silages. *Lactobacillus plantarum* was found in BG and BGT materials, and appeared to survive only when BG was ensiled alone. Similar to Exp. 1, *L. buchneri* was not detected in either BG or BGT material, but was detected after 14 d of storage in both BG and BGT silages. *Streptococcus* spp. were found only in SC silage, whereas *Pediococcus acidilactici* was detected in BG and BGT silages.

DISCUSSION

Lactic acid production was low in SC silage and the content decreased with prolonged ensiling (Exp. 2). Lactic acid content was also lower in BG silage after 56 than after 14 d of storage. It has been shown that good preservation is difficult to obtain with SC ensiled alone (Niwa, 2001), and lactic acid can predominate in BG silage, especially with a short storage period (Schneider et al., 1995; Nishino et al., 2003a). A reduction in lactic acid content coupled with an increase in acetic acid content was shown in SC and BG silages, suggesting that *L. plantarum* or *L. buchneri* metabolism was activated. *Lactobacillus plantarum* and *L. buchneri* are known to produce acetic acid from lactic acid under anaerobic conditions (Lindgren et al., 1990; Oude Elferink et al., 2001), and the 2 species were detected in BG

Table 3. Chemical and microbial composition of wet by-products and their TMR silages prepared with soybean curd residue and brewers grains (experiment 2)

Item	Treatment[1]				SEM	Analysis of variance[2]		
	SC	BG	SCT	BGT		M	S	M × S
Ensiling for 14 d								
pH	4.38	3.49	3.83	3.98	0.02	**	NS	**
Lactic acid (%)	0.52	1.01	2.23	1.57	0.08	NS	**	**
Acetic acid (%)	0.19	0.09	0.62	0.50	0.04	*	**	NS
Propionic acid (%)	0.00	0.00	0.01	0.00	0.01	NS	NS	NS
Butyric acid (%)	0.00	0.00	0.00	0.00	—	—	—	—
Undissociated acids[3] (%)	0.26	0.80	1.71	1.10	0.05	NS	**	**
Ethanol (%)	0.05	0.72	0.22	1.41	0.05	**	**	**
1,2-Propanediol (%)	0.00	0.00	0.00	0.01	0.00	NS	NS	NS
Lactic acid bacteria (log cfu/g)	8.30	6.94	7.94	7.93	0.10	**	*	**
Yeasts (log cfu/g)	5.49	5.92	3.92	5.56	0.43	**	NS	NS
Ensiling for 56 d								
pH	4.60	3.14	3.90	4.00	0.05	**	NS	**
Lactic acid (%)	0.22	0.69	1.32	1.12	0.05	*	**	**
Acetic acid (%)	0.50	0.25	0.31	0.45	0.12	NS	NS	NS
Propionic acid (%)	0.12	0.01	0.02	0.01	0.01	**	**	**
Butyric acid (%)	0.00	0.10	0.00	0.00	0.08	NS	NS	NS
Undissociated acids[3] (%)	0.32	0.73	0.90	0.85	0.11	NS	*	NS
Ethanol (%)	0.21	0.55	0.42	0.72	0.11	*	NS	NS
1,2-Propanediol (%)	0.02	0.00	0.00	0.03	0.00	*	*	**
Lactic acid bacteria (log cfu/g)	8.24	7.08	4.78	5.95	0.29	NS	**	**
Yeasts (log cfu/g)	<2.00	5.20	<2.00	2.46	—	—	—	—

[1]SC = soybean curd residue; BG = brewers grains; SCT = TMR containing SC; BGT = TMR containing BG.

[2]M = effect of by-product material; S = effect of storage method.

[3]Sum of undissociated lactic acid and VFA.

*$P < 0.05$; **$P < 0.01$; NS = $P > 0.05$.

silage. Secondary fermentation associated with clostridia could also be involved in the reduction of lactic acid in SC silage, because butyric acid was determined after 56 d of ensiling.

Lactic acid fermentation was enhanced in ensiled TMR. Unlike silages stored alone, lactic acid production was greater in SC compared with BG. Although fermentable sugars were increased by mixing with other feeds in the 2 TMR mixtures, yeasts might have grown as well as LAB in BGT silage. Yeast numbers were lower in SCT than BGT silage after 14 d of storage, suggesting that use of leguminous feed might have suppressed silage yeasts. Muck and O'Kiely (1992) reported that substances inhibiting yeasts may be produced during ensiling and retard aerobic spoilage in legume crop silage. *Lactobacillus buchneri* was found in BGT but not in SCT silage. However, the bacterium could not account for enhanced ethanol fermentation in BGT silage, because alcoholic fermentation was shown to be suppressed when *L. buchneri* was inoculated (Nishino et al., 2007).

Aerobic deterioration occurred promptly in fresh products and silages stored alone, confirming that silage fermentation was necessary to prevent spoilage in TMR silage. In conventional crop silages, yeast growth may trigger aerobic deterioration (Hara et al., 1979), and silages with yeast counts >10^5 cfu/g are prone to deteriorate after silo opening (McDonald et al., 1991). This could explain the instability of SC and BG silages, but not the stability of 14-d TMR silages. We have reported the same property of BGT silage: aerobic deterioration was not found even when a high yeast population survived (Nishino et al., 2004; Nishino and Hattori, 2007; Wang and Nishino, 2008). The SCT silage also showed high stability regardless of storage period, but it was unclear whether this stability was related to yeast counts. Yeast counts were <10^4 cfu/g in 14-d SCT silage, indicating that a low number of undesirable microorganisms might account for this stability. Even so, regardless of by-product type, prolonged ensiling could further improve the resistance to aerobic deterioration, because yeasts became almost or completely undetectable in 56-d TMR silages.

Culture-independent analysis is considered to have less bias than the conventional plate-count technique (Ercolini, 2004) and is increasingly being used to understand the make-up of the community of silage microorganisms (May et al., 2001; Yang et al., 2006). In this study, *Weissella* spp. and *L. brevis* were common in the 2 TMR silages, and *L. buchneri* was found exclusively in BGT silage. *Weissella* spp. were also found in fresh SC product, but *L. brevis* and *L. buchneri* were not
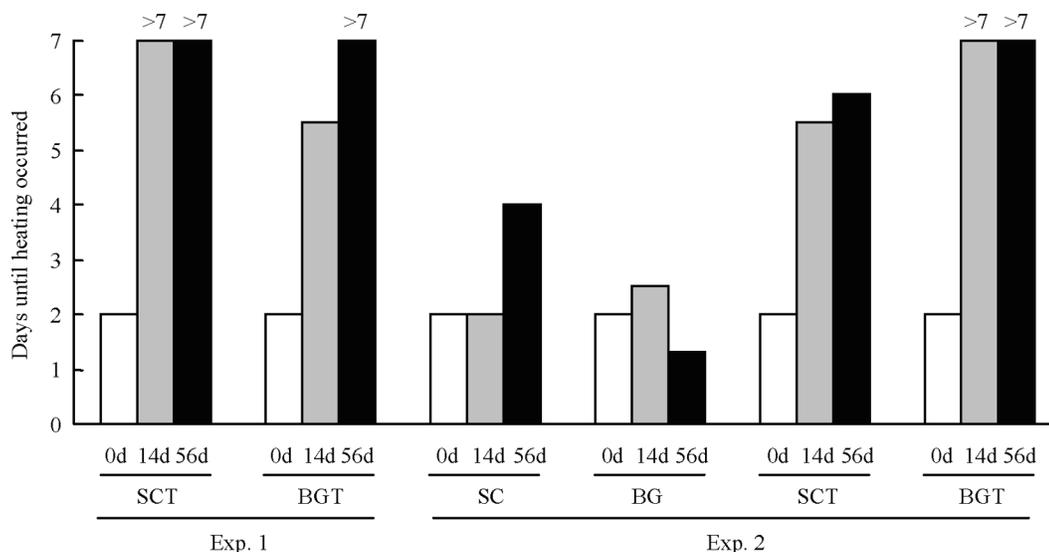
Figure 1. Aerobic stability of wet by-products and their TMR mixtures ensiled for 0, 14, and 56 d. Changes in temperature were manually recorded at 12-h intervals. SC = soybean curd residue; BG = brewers grains; SCT = SC-containing TMR silage; BGT = BG-containing TMR silage.

detected in either wet by-products or their TMR mixtures. These results suggest that heterofermentative LAB were predominant during fermentation of TMR silage, although there was no indication why this is the case. *Lactobacillus buchneri* has been shown to activate at later stages of ensiling (Driehuis et al. 1999), but in BGT silages LAB were detected from 14 d of ensiling. Because 1,2-propanediol, a specific metabolite of *L. buchneri* in silage, was increased by prolonged ensiling, the metabolic activity of *L. buchneri* in BGT silage, even if detected, may have been dormant in the early stages of ensiling. It is difficult to explain why *L. buchneri* emerged exclusively in BGT silage. However, in a subsequent experiment in which TMR silages were prepared with the same recipe as in this study, 1 (3%) and 13 (43%) isolates from 30 colonies were identified as *L. buchneri* in SCT and BGT silages, respectively (our unpublished data). *Lactobacillus buchneri* was also found in 56-d BG silage, suggesting that BG might contain factors or substances that could facilitate growth and survival of *L. buchneri*. In contrast, *L. plantarum* disappeared in TMR silages even though it was detected in material mixtures. *Lactobacillus plantarum* is one representative LAB frequently found in well-preserved silages (McDonald et al., 1991), but this homofermentative LAB might have difficulty surviving during fermentation of low-moisture TMR silages.

Although 3 heterofermentative LAB (*Weissella* spp., *L. brevis*, and *L. buchneri*) were assumed to be involved in silage stability, only *L. buchneri* was shown to actively suppress aerobic spoilage (Driehuis et al., 1999;

Weinberg et al., 1999; Ranjit and Kung, 2000). The stability of BGT silage could thus be accounted for by *L. buchneri* activity, but the stability of SCT silage is difficult to explain by the bacteria revealed by PCR-DGGE. Neither *Weissella* spp. nor *L. brevis* improved stability when inoculated alone (Adesogan et al., 2003; Danner et al., 2003), and no metabolites were found that could inhibit the growth of yeasts and molds. There might have been synergism between *Weissella* spp. and *L. brevis*, but this would need to be confirmed by inoculation with aerobically unstable silages. In another experiment, we isolated *Weissella* spp. and *L. brevis* from stable TMR silages (our unpublished data), and further studies will be conducted to ascertain any possible synergistic effect.

CONCLUSIONS

Resistance to aerobic deterioration can be expected in TMR silages regardless of the wet by-products (SC and BG) formulated as principal ingredients. Lactic acid production was greater in SCT than in BGT silage, but resistance to spoilage appeared unrelated to the composition of fermentation products. Yeasts became undetectable when ensiling was prolonged, further improving stability in both SCT and BGT silages. Bacterial communities seemed to be established after 14 d of storage, although many indigenous bacteria did not survive, especially in TMR silages. *Weissella* spp. and *L. brevis* were common in stable TMR silages, whereas *L. buchneri* was detected only when BG was used. The
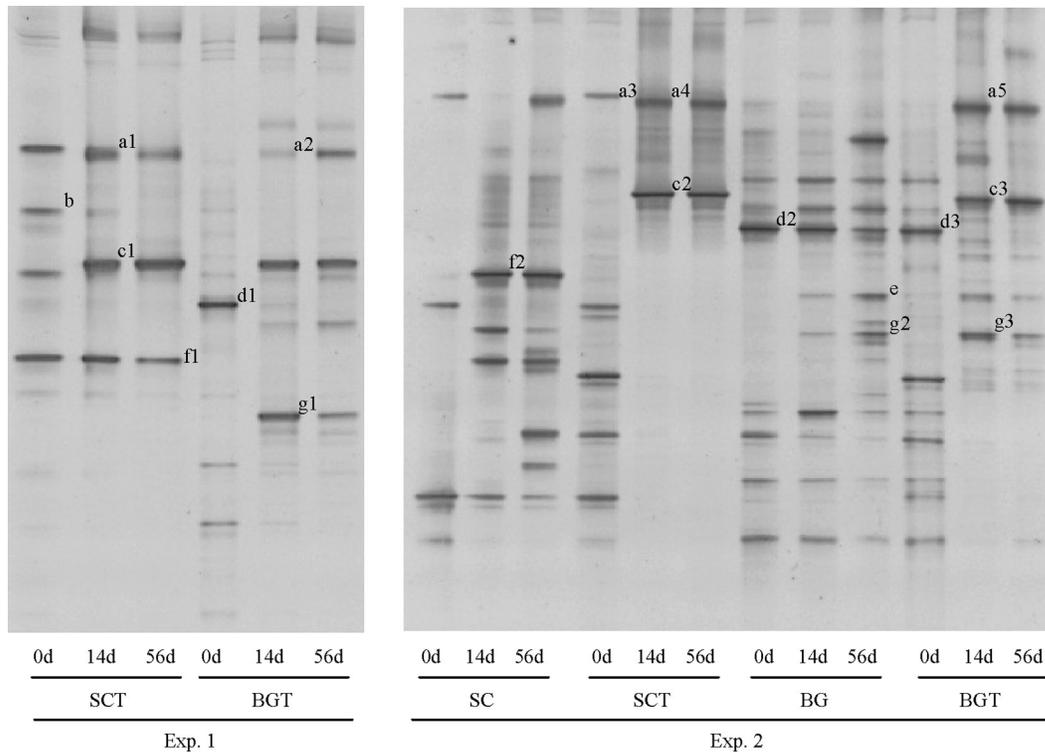
Figure 2. Bacterial communities in wet by-products and their TMR mixtures ensiled for 0, 14, and 56 d. Denaturing gradient gel electrophoresis was carried out at a constant voltage of 150 V for 12 h at 60°C, and several DNA bands were excised and sequenced. SC = soybean curd residue; BG = brewers grains; SCT = SC-containing TMR silage; BGT = BG-containing TMR silage. a1 to a5 = *Weissella* spp.; b = *Lactobacillus fermentum*; c1 to c3 = *Lactobacillus brevis*; d1 to d3 = *Lactobacillus plantarum*; e = *Pediococcus acidilactici*; f1 to f2 = *Streptococcus* spp.; g1 to g3 = *Lactobacillus buchneri*.

results suggest that *L. buchneri* was involved in the stability of BGT silage, but it is not clear which bacteria were involved in the stability of SCT silage.

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