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Effect of a Commercial Inoculant on the Fermentation Quality of ABP Silage in Thailand

Sadahiro OHMOMO[1]*, Masaharu ODAI[2], Pimpaporn PHOLSEN[3], Sunee NITISINPRASERT[4], Damrussiri KRAYKAW[5] and Supanit HIRANPRADIT[6]

[1,2] Animal Production and Grassland Division, Japan International Research Center for Agricultural Sciences (JIRCAS) (Tsukuba, Ibaraki 305–8686, Japan)
[3] Khon Kaen Animal Nutrition Research Center, Department of Livestock Development, Ministry of Agriculture and Cooperatives, Thailand (Tha Phra, Khon Kaen 40260, Thailand)
[4] Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University (Chatuchak, Bangkok 10900, Thailand)
[5] Thailand Office, Japan International Research Center for Agricultural Sciences (JIRCAS)(Chatuchak, Bangkok 10900, Thailand)
[6] Plant Pathology and Microbiology Division, Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand (Chatuchak, Bangkok 10900, Thailand)

Abstract

The effect of a commercial inoculant on the fermentation quality of silage made from agricultural by-products (ABP silage) prepared in Thailand was examined. By adding an inoculant, the pH of silage became low and the counts of yeast and clostridia in silage decreased in comparison with the silage without inoculant. The effect of temperature against the inoculant was also examined by using a modified pouch method. The addition of inoculant provided good quality silage at 30ºC. However at 45ºC, the addition of the inoculant resulted in a remarkable decrease in the amount of lactic acid produced and an increase in pH about 0.6–0.8 with no good quality silage produced. From these results, it was suggested that the inoculant used in this study was effective for improving the fermentation quality of ABP silage in Thailand at temperatures less than 37ºC, but was not effective at 45ºC.

Discipline: Animal industry
Additional key words: lactic acid bacteria

Introduction

Feeding of good quality silage seems to be a suitable way to increase raw milk production (RMP) in spite of not having wide use in Thailand. Actually, a project[6] supported by Japan International Cooperative Agency reported that feeding of good quality silage throughout a year stably increased the average RMP to a level which was about 1.5 times higher than that of Thailand[4]. However, making good quality silage in Thailand is not always assured. Therefore, lactic acid bacteria (LAB) strains to make good quality silage in tropical regions were screened in Thailand[2].

On the other hand, LAB strains as silage additives (inoculants) have already been commercialized in cold and temperate zones and are a big market[3]. It has not been tested whether those inoculants are suitable or not for making good quality silage in Thailand. Namely, it is necessary to examine the ability of commercial inoculants by preparing actual silage. Therefore, test-scale ABP silage were prepared with a typical commercial inoculant in Thailand and the ability of the inoculant was evaluated by using a modified pouch method adaptable to tropical conditions.

In this paper, the fermentation quality of ABP silage prepared with a commercial inoculant and changes in silage microorganisms (LAB mainly from the inoculant, coliform bacteria: CFB and yeast) examined in the modified pouch method[5] are summarized.

Present address:
[2] Department of Research Planning and Coordination, National Institute of Livestock and Grassland Science (Tsukuba, Ibaraki 305–0901, Japan)
*Corresponding author: fax +81–29–838–6653; e-mail bupmomo@jircas.affrc.go.jp
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Materials and methods

1. Silage

Silage material consisted of 35% raw sugar cane (w/w), 35% of dried rice straw (w/w) and 30% of raw beer waste (w/w). Sugar cane used in this experiment contained 15–16 Brix % of sugar in fresh matter. The dry matter (DM) content of rice straw and beer waste were about 85% and 15%, respectively. The mixed silage material (30 kg) was put into a plastic bag with (sample number S2 and S3) or without (sample number S1) inoculant and air was removed with a vacuum suction machine, then wrapped with plastic film (Fig. 1). Each silage was kept outdoors without direct sunshine (temperature: 30–35ºC). All operations were done at Khon Kaen Animal Nutrition Research Center, Tha Phrae, Khon Kaen, NE-Thailand.

2. Inoculant

Snow Lact L, which was supplied by the courtesy of Snow Brand Seed Co., Ltd. (Sapporo, Japan), was used as the inoculant for silage-making. This inoculant consisted of the living cells of *Lactobacillus rhamnosus* and is one of the useful Japanese inoculants evaluated as a possible product for higher temperature conditions among the many LAB inoculants. Five grams of the inoculant suspended in 1 L water was sprayed into 1 t of material. This addition ratio of the inoculant makes the LAB density 10^5 cfu/g of fresh material.

3. Pouch method to evaluate LAB strain for silage-making

The medium of the modified pouch method[5] used in this study consisted of 1–2 cm lengths of cut Napiergrass which was cultivated for 30 days, dried and autoclaved then adjusted to a moisture content of 75% with the addition of 1.5% glucose (w/w). The medium (40 g) inoculated with silage microorganisms at the designated inoculum size was put into a plastic bag with a double film of nylon and polyethylene (Hiryu KN 210, Asahi-Kasei Co., Japan, 20 × 30 cm, 0.1 mm thickness, oxygen permeability 1 mL/m^2/day), sealed by a vacuum sealer machine and incubated for 24 h and 48 h. As silage microorganisms, strain SG 2-1 Y (tentatively identified as *Saccharomyces cerevisiae*) and strain SG 1-1T (tentatively identified as *Enterobacter* sp.) were used as yeast and CFB, respectively. All of experiment were done in duplicate.

Fig. 1. Plastic bag sealed and wrapped after removing air

4. Microbiological analysis

The number of microorganisms in silage was enumerated by the plate culture count method. Silage samples (10 g) were shaken well with 90 mL of sterilized distilled water, and 10^{-1}–10^{-6} serial dilutions were made in a 0.85% sodium chloride solution. LAB were counted on an agar plate of lactobacilli MRS broth (Difco, USA) after incubation in an anaerobic box (Mitsubishi Gas Chemical Co., Inc., Japan) at 45ºC. CFB were counted on a plate of violet red bile agar with lactose (Difco, USA) after incubation at 37ºC. Yeast were counted on a plate of potato-dextrose agar (Nissui Seiyaku Ltd., Japan) after incubation at 30ºC. Clostridia were counted on a plate of sulfite iron agar base (Merck, Germany) after incubation at 37ºC for 24 h using sample solution autoclaved at 121ºC for 15 min. Colonies were counted after 24 and 48 h culture and their numbers were expressed as viable numbers of microorganisms in colony-forming units (cfu) per gram of fresh silage.

5. Lactic acid analysis

After the culture, 10 g of silage was suspended into 30 mL of distilled water and kept at 4ºC for 2 h to make a sample solution for lactic acid analysis. Amount of lactic acid produced was analyzed by using high performance liquid chromatography (HPLC) (Thermo Quest Spectra P-100) with an organic acid analysis column Aminex HPX-87H (Bio-Rad, diameter 7.8 mm × length 300 mm) at 45ºC. Eight mM sulfuric acid solution was used as a mobile phase with a speed of 0.5 mL/min and an UV/VIS photometer (Thermo Quest Spectra UV-150) at wave length 230 nm was used as a detector. Tartaric acid was used as an internal standard acid.

Results and discussion

1. The count of microorganisms in silage

The ABP silage prepared (Fig. 2) had a very good smell and good palatability for cattle. The count of

Fig. 2. ABP silage after 2-weeks fermentation

microorganisms in silage are shown in Table 1. During the first two days, each of the microorganisms in silage grew independently (S2 in Table 1). After that, the addition of LAB (S3 in Table 1) smoothly accelerated the lactic acid fermentation and rapidly made the conditions acidic. The silage material contained about 5.0–5.5% of sugar (5.25–5.60 Brix % in fresh silage) and LAB were able to utilize enough sugar in spite of the vigorous growth of CFB, yeast and clostridia in the early stage of the fermentation. As a result, the growth of CFB, yeast and clostridia were inhibited and the cell count after two weeks were extremely reduced. The count of LAB was also reduced, but the degree of reduction was little. From this result, it is judged that the addition of inoculant was effective in improving the fermentation quality of silage, at least at temperatures of about 30–35ºC. However, if the silo containing silage is exposed directly to sunshine during the silage fermentation process, the count of microorganisms in silage may be affected by the increased temperatures in the silo. Therefore, changes in the counts of each of the microorganisms in silage at 45ºC were examined as well as at 30 and 37ºC by using the modified pouch method which is an evaluating system[5] of LAB for silage preparation with different inoculum of typical silage microorganisms (LAB, CFB and yeast) in Thailand.

2. Changes in typical silage microorganisms in a pouch method at various temperatures

Changes in LAB, CFB and a yeast strain as typical silage microorganisms during silage fermentation were examined by using the modified pouch method. The number of microorganisms on forage crops and grasses is variable and generally enumerated at the level of 10^2–10^5 cfu/g[1]. Furthermore, the addition of commercial inoculant at the level of 10^5 cfu/g is recommended for preparing good quality silage[3]. Therefore, the inoculum size of each microorganism was decided to be 10^5 or 10^2 cfu/g. The results are shown in Table 2. In any silage cultured at 30 and 37ºC, the pH dropped down to less than 4.6 and LAB was the dominant microorganism. However in the

Table 1. Counts of microorganisms in ABP silage prepared in Thailand

Sample no.	Addition of inoculant	Time of fermentation (d)	Final pH	Counts of microorganisms (cfu/g)			
				LAB	CFB	Yeast	Clostridia
S 1	–	14	4.26	2.3×10^7	9.3×10^6	5.4×10^6	3.2×10^6
S 2	+	2	4.47	8.9×10^7	5.6×10^6	7.2×10^7	4.1×10^6
S 3	+	14	4.08	1.5×10^6	3.8×10^5	6.5×10^2	6.3×10^2

Table 2. Effect of inoculum size on the growth of microorganisms in a pouch method

Inoculum size	Temperature (ºC)	pH after		Colony counts after 24 h (cfu/g)			Colony counts after 48 h (cfu/g)		
		24 h	48 h	LAB	Yeast	CFB	LAB	Yeast	CFB
A	30	4.28	4.32	1.5×10^7	2.2×10^5	3.8×10^6	9.0×10^6	1.7×10^6	4.6×10^6
	37	4.34	4.53	2.1×10^7	3.0×10^5	3.2×10^6	7.2×10^6	1.8×10^5	5.8×10^6
	45	4.94	5.05	4.8×10^5	1.0×10^5	1.0×10^5	1.5×10^6	8.0×10^5	1.6×10^6
B	30	4.46	4.60	6.7×10^6	1.1×10^6	7.8×10^6	4.7×10^6	3.1×10^6	6.2×10^6
	37	4.59	4.32	1.5×10^7	6.8×10^5	2.2×10^6	7.6×10^6	5.8×10^5	7.5×10^6
	45	5.31	5.17	4.2×10^5	6.8×10^6	4.5×10^6	2.4×10^6	1.1×10^7	4.4×10^7

Inoculum size A(cfu/g): LAB(10^5), yeast (10^2) and CFB(10^2). Inoculum size B(cfu/g): LAB(10^5), yeast (10^5) and CFB (10^5). LAB: *Lactobacillus rhamnosus* which was isolated from inoculant (Snow Lact L) commercialized in Japan by using MRS broth. Yeast: *Saccharomyces cerevisiae* SG2-1Y. CFB: *Enterobacter* sp. SG1-1T.

Table 3. Effect of culture temperature on the production of lactic acid

Inoculum size	Temperature (ºC)	Lactic acid produced (mg/g)	
		at 24 h culture	at 48 h culture
A	30	6.62	5.16
	37	7.00	2.84
	45	4.00	2.54
B	30	4.84	4.21
	37	3.50	4.52
	45	1.92	2.48

Inoculum size A(cfu/g): LAB(10^5), yeast (10^2) and CFB(10^2).
Inoculum size B(cfu/g): LAB(10^5), yeast (10^5) and CFB (10^5).

culture at 45ºC, the growth of LAB was repressed and the pH was about 5.0. Further, the lactic acid fermentation was repressed by the large inoculum size of CFB and yeast and the pH was about 5.2–5.3. Lactic acid fermentation was also repressed at 45ºC, and the amount of lactic acid produced at 45ºC at 24 h culture was about 50–60% of that produced at 30 and 37ºC (Table 3).

From these results, it is clear that the commercial inoculant has no ability to inhibit directly the growth of CFB and yeast strains used in this experiment. However, the silage prepared with the commercial inoculant was of good quality and showed no high levels in the counts of CFB, yeast and clostridia. This phenomenon should be understood in that the addition of commercial inoculant gives a high level of LAB population in silage, it accelerates the lactic acid fermentation, and it quickly reduces the silage pH to about 4.0–4.5. In addition, the use of this inoculant in Thailand seems to have an advantage for making good quality silage so far as the silo is kept at less than 35ºC. However, a silo exposed directly to sunshine should be avoided because the temperature of silage in the silo will increase to more than 37ºC which is not suitable for this inoculant.

From these considerations, it is necessary to develop thermotolerant LAB strains for making good quality silage in Thailand. The modified pouch method can be used effectively for the purpose of screening thermotolerant LAB strains.

Conclusion

1. The inoculant Snow Lact L was effective in making good quality silage in Thailand at temperatures under 35ºC.
2. *Lactobacillus rhamnosus* isolated from the commercial inoculant showed powerful lactic acid fermentation at 30 and 37ºC, but it was strongly repressed at 45ºC.

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