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Attempt to Practical Use of *Lactobacillus plantarum* SP 1-3 in Spray Dried Granule Form for Making Good Quality Silage in Thailand

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ABSTRACT

For the practical application of spray-dried granule of *Lactobacillus plantarum* SP 1-3 isolated from a corn silage prepared in Thailand, a low-cost and convenient culture medium formulated from available materials in Thailand was designed for the large scale culture. The best formulation of culture medium was 2% molasses, 0.5% rice bran, 0.2% yeast extract, 0.2% Na-acetate, 0.05% KH_2PO_4, 0.02% $MgSO_4$ and 0.01% $MnSO_4$. $4H_2O$ and was used for cultivating spray-dried granule strain SP 1-3. This SP 1-3 granule was stable and kept as the living cell count of $10^{8\sim9}$ cfu/g in storing at 4°C for 28 days and surely improved the fermentation quality of Napier grass silage. To use this granule for the preparation of practical scale silage, the low-cost and convenient method to increase the cell mass of Lactic acid bacteria (LAB) strain was developed by using a 5-liter plastic bottled drinking water. The culture solution of SP 1-3 obtained by this method was named "PBS-SP". The fermentation qualities of Pangola grass and Erianthus silages inoculated with PBS-SP were improved effectively. However, the inoculation of LAB strain has no effect on the reduction of living yeast cell in silage and aerobic deterioration of silage by yeast was remained as a problem to be solved.

Key words: silage, silage inoculant, *Lactobacillus plantarum*

INTRODUCTION

The low raw milk production in Thailand trends to be a problem of dairy farming as well as the livestock industry, even the amount of milk and fermented milk consumption is increasing from year to year (Ohmomo *et al.*, 2002a). It is recognized that feeding of good quality silage is an effective and easily adaptable technique for increasing and stabilizing the quantity of raw milk production despite its limited application in Thailand. However, successful results of

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producing good quality silage does not always ensure if ensilage depends on natural fermentation. The instability of fermentation quality is mainly attributed to the ability of lactic acid bacteria (LAB) as well as the low content of water soluble carbohydrates (WSC) in tropical pasture grasses (Kawamoto, 1999). The lack of WSC is easily supplied by adding molasses. But LAB strains having the ability suitable for silage fermentation in tropical regions are not always inhabitable. Therefore, a LAB strain SP 1-3 was screened for stabilization of good quality silage production in Thailand (Ohmomo *et al*., 2004). At the same time, the development of low-cost and convenient culture medium was required to harvest large amount of LAB cells for the practical use of strain SP 1-3 by farmers because it is well known that LAB strains are usually cultured in high nutrient broth adapted for their requirement of vitamins, amino acids, etc and consequently high cost of LAB cells production. Based on the results of screening and elucidation of culture medium, a new silage inoculant was prepared from spray-dry *Lactobacillus plantarum* SP 1-3 and the trial silages were prepared by using this new silage inoculant.

In this paper, the result of shelf life of LAB dry cell granule by the spray-dry method together with the development of a low-cost and convenient culture method is summarized. Further the fermentation quality of silage inoculated with this new silage inoculant is also proposed.

MATERIALS AND METHODS

1. Microorganism

A LAB strain SP 1-3 was used in this study. This strain was isolated from the corn silage prepared in Nakhon Pathom Province, Thailand, and tentatively assigned to *Lactobacillus plantarum* (Ohmomo *et al.*, 2004).

2. Preparation of analysis silage sample

Ten grams of silage was used for the determination of dry matter content (105°C for 48 h). Another 10 g of silage was suspended into 30 ml of distilled water and kept at 4°C for 24 h to make a sample solution for acids analysis by using high performance liquid chromatograph (HPLC). Further 10 g of silage was mixed 30 ml of sterilized saline water to make a sample solution for enumeration of microorganisms. Sampling of silage was set in four points of each silage bale (n=8).

3. Microbial enumeration

The number of microorganisms was enumerated by the plate culture count method using lactobacilli MRS broth agar (Merck, Germany) for LAB, violet red bile agar with lactate (Difco, USA) for coliform bacteria (CFB) and potato-dextrose agar (Nissui Seiyaku Ltd., Japan) for yeast, respectively. The plate was cultured for 24 h at 45, 37, 30°C for determination of LAB, CFB and yeast, respectively. Colonies were counted and their numbers were expressed as visible numbers of microorganisms in colony-forming unit (cfu) per gram of fresh matter or ml of culture broth.

4. Chemical analysis and HPLC

Dry matter content was measured by drying at 60°C for 48 h. Volatile basic nitrogen (VBN) content was determined by the diffusion method using Conway's unit (Cai, 1999). Lactic acid and other volatile fatty acids (VFA) such as acetic acid, propionic acid and n-butyric acid in silage were analyzed by using a HPLC (Thermo Quest Spectra P-100, UK) with an organic acid analysis column Aminex HPX-97H (Bio-Rad, USA, diameter 7.8 mm × length 300 mm) at 45° C. Eight mM of sulfate 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, UK) at wave length 230 nm was used as a

detector. Tartaric acid was used as an internal standard.

5. Spray-dry method

A strain SP 1-3 was cultured at 45°C for 24 h by using the coconut juice prepared from the crushed coconut jelly waste (adjusted pH to 6.5). After that, 450 g of the powder mixture of skim milk and dextrin (3:1, w/w) was added to the culture (3.0 L) and the culture was used for preparing dry cell granule of strain SP 1-3 by spray-dry method. A laboratory scale spray dryer (Heto FD4, Belgium) was used to process samples at a constant air inlet of 170°C. The feed solution was atomized into the drying chamber using a two-fluid nozzle and the product was dried with a very low residence time. The outlet temperature was maintained at 80°C, in order to obtain granule with less than 4% moisture content. The living cell count of SP 1-3 in the dry cell granule (570 g) from receiver chamber was 6.08×10^8 cfu/g while the one from nozzle chamber was 1.87×10^9 cfu/g.

6. Silage preparation

Laboratory scale Napiergrass silage was prepared as follows: Napier grass, (i) harvested in Pathum Thani province at the growth stage of about 30-40 cm height and dried for one day under sunshine, (ii) was cut into about 2 cm length, (iii) inoculated with 1 g of spray-dried granule to 1 kg of material and (iv) put into an airtight plastic pouch together with 1.5% glucose. The pouch was sealed by a vacuum sealer and incubated at 45°C , anaerobically. On 1-day, 7-day, 14-day and 28-day incubation, the pouch was opened and the counts of microorganisms and silage pH were analyzed.

Practical-use scale silage was prepared as follows: Pangola grass (*Digitaria decumbens* Stent), harvested and dried for one day under sunshine, was cut into about 3-5 cm lengths, inoculated with LAB solution arranged from spray-dried granule (10^6 cfu/g of material) and put into a plastic bag (about 20 kg in capacity 60-liter bag) with 2% of molasses solution (equivalent to 1% WSC addition). The plastic bag was sealed by a vacuum sealer and wrapped with plastic film to make a roll bale. The bale kept at room temperature. Erianthus (*Erianthus* sp.) was also used as a silage material together with rice straw (added about 10%, w/w). But no molasses was added because of rich sugar content in Erianthus. Practical-use scale silages were prepared at Khon Kaen Animal Nutrition Research and Development Center, Tha Phra, Khon Kaen, NE-Thailand. The method of silage preparation from Erianthus was the same with the case of Pangola grass silage.

LAB solution was prepared as follows: 200 mg of spray-dried granule and 130 g of nutrient powder (molasses 2%, rice bran 0.5%, yeast extract 0.1%, Na-acetate 0.2%, KH_2PO_4 0.05%, $MgSO_4 \cdot 7H_2O$ 0.02%, $MnSO_4 \cdot 4H_2O$ 0.01%) were put into a 4.5-liter plastic bottled drinking water and mixed, then the plastic bottle was kept for 24 h at room temperature.

After storing for 90 days, the bale was opened and the counts of microorganisms, silage pH and the amounts of lactic acid and volatile fatty acids (VFA) in silage were analyzed. All experiments were conducted in triplicate.

RESULTS AND DISCUSSION

1. Culture conditions of strain SP 1-3

1-1) Effect of culture temperature on the LAB cells growth and lactic acid production

To harvest large amount of LAB cells by a low-cost and convenient culture method, effect of culture temperature on the growth of LAB strain SP 1-3 was examined. On the anaerobic culture using lactobacilli MRS broth for 24 h, the maximal cell count (ca. 6.40×10^9 cfu/ml) and maximum lactic acid production (ca. 6.15 mg/ml) were obtained at 30°C and 45°C, respectively as shown in Table 1. Almost the same level for good growth

and well production of lactic acid on the culture at 30~45°C was reasonable because this strain was selected under the cultivation at 45°C . On further experiment, this strain is cultured at 45°C.

1-2) Effect of initial medium pH on the LAB cell growth and lactic acid production

The effect of initial medium pH on the growth of strain SP 1-3 and its lactic acid production were examined because the initial medium pH sometimes becomes an important factor for the initial growth of LAB strains. Unexpectedly, the effect of initial medium pH for this strain was not remarkable. The culture on the medium pH between from 5.0 to 7.0 resulted in the almost same level of both maximal lactic acid production (5.2~6.2 mg/ml) and cell growth (10^9 cfu/ml).

2. Development of microbial medium formulation for the culture of strain SP 1-3

2-1) Effect of carbon and nitrogen sources on the growth and lactic acid production

To compose the culture medium instead of MRS broth which is very expensive and hard for practical use, various carbon sources obtained with low-cost in Thailand were examined. Molasses, sucrose and glucose resulted in the maximal cell count of 1.2×10^9 -2.1×10^9 cfu/ml and lactic acid production of 4.6-6.7 mg/ml as shown in Table 2. Molasses is the most common carbon source in the fermentation industry and its cost in Thailand is very low. Raw cassava chips and corn granules are also low-cost carbon sources in Thailand. However, the amount of lactic acid production was very low level of 0.5-0.58 mg/ml. It is understood that main saccharide of these sources is starch which is hardly fermentable for LAB strains.

Considering the nitrogen source, it was found that rice bran or soy bean waste gave the high range of cell count between 2.2×10^8 to 3.0×10^8 cfu/ml and lactic acid production of 2.1 to 2.2 mg/ml as shown in Table 2. These were inferior to that of peptone and meat extract. However, it is convenient to find rice bran in Thailand at low-cost and is able to contribute to yield enough cell concentration of 3.0×10^8 cfu/ml.

2-2) Effect of nutrient additives on the growth and lactic acid production

To increase the cell concentration in the medium using rice bran as a nitrogen source, some other enrich additives of yeast extract, coconut milk, coconut juice and corn steep liquor were examined as shown in Table 3. The satisfied results were obtained by the addition of 0.2% yeast extract together with 0.5% rice bran (maximal cell count: ca. 2.3×10^9 cfu/ml, lactic acid production: ca. 4.2 mg/ml). The price of yeast extract is not so low. However, the grade of yeast extract is varying. The price is reduced if the grade of food additive is selected.

From these results, the low-cost culture medium to harvest large amount of LAB cells was proposed as follow: molasses 2%, rice bran 0.5%, yeast extract 0.2%, Na-acetate 0.2%, minerals (Mg^{++}, Mn^{++}), pH 6.5. By the anaerobic culture of strain SP 1-3 using this medium at 35°C for 24 h, the cell count of 10^9 cfu/ml is able to be expected.

3. Yield and LAB survival in dried granule by spray dry technique

LAB dry cell granule was prepared from 3 litres of culture solution by the spray-dry method according to the method mensioned. The cell concentration of SP 1-3 in the culture solution was 1.26×10^9 cfu/ml. After addition of skim milk and dextrin used as carriers, the cell concentration was changed to be 6.05×10^8 cfu/ml and was equivalent to 4.03×10^9 cfu/g. By spray-dry technique, 570 g of cell granule was obtained and provided the concentration of 6.08×10^8 cfu/g in the reciever chamber. The survival rate of the LAB

Table 1 Effect of culture temperature on the growth of strain SP 1-3.

Culture temperature (°C)	pH	Cell counts (cfu/ml)	Lactic acid produced(mg/ml)
30	3.71	1.27×10^9	5.04
35	3.61	6.40×10^9	6.07
40	3.64	1.18×10^9	5.46
45	3.66	1.32×10^9	6.15
50	5.18	3.20×10^9	0.57

Strain SP 1-3 was anaerobically cultured for 24 h at various temperatures by using MRS broth.

Table 2 Effect of carbon sources and nitrogen sources on the growth of strain SP 1-3.

Carbon and nitrogen sources (%)	pH	Cell counts (cfu/ml)	Lactic acid produced (mg/ml)
Carbon source			
Glucose (1.0%)	3.45	1.38×10^9	6.40
Sucrose (1%)	3.46	1.18×10^9	6.68
Molasses (2.0%)	3.52	2.07×10^9	4.57
Sugar cane juice (10%)	3.68	9.4×10^8	5.78
Raw cassava chips (5.0%)	4.62	8.7×10^8	0.50
Raw corn granule (5.0%)	4.82	3.7×10^8	0.58
Nitrogen source			
NH_4Cl (0.2%)	4.15	7.0×10^7	1.82
$NaNO_3$ (0.4%)	4.41	7.0×10^7	0.82
Peptone (0.5%)	3.61	7.3×10^8	3.34
Meat extract (0.5%)	3.34	4.0×10^9	6.43
Soy bean waste (1.0%)	4.03	2.2×10^8	2.23
Rice bran (1.0%)	3.92	3.0×10^8	2.11

Strain SP 1-3 was cultured for 24 h at 45°C anaerobically by using a medium consisted of carbon source, nitrogen source, Na-acetate 0.2%, $MgSO_4.7H_2O$ 0.02%, $MnSO_4.4H_2O$ 0.01% and pH 6.5. In the case that various carbon sources were studied, 0.5% peptone wa

Table 3 Effect of nutrient additives on the growth of strain SP 1-3.

Nutrient additives[1]					pH	Cell counts	Lactic acid produced
RB	YE	CM	CJ	SCL		(cfu/ml)	(mg/ml)
+	-	-	-	-	3.87	9.3×10^8	2.48
+	+	-	-	-	3.46	2.26×10^9	4.19
+	-	+	-	-	3.83	9.7×10^8	2.37
+	-	-	+	-	3.99	4.8×10^8	1.53
+	-	-	-	+	4.08	5.0×10^8	1.91

[1] Additives: RB (Rice bran, 0.5%), YE (Yeast extract, 0.2%), CM (Coconut milk, 0.5%), CJ (Coconut juice, 10%), SCL (Steepel Corn liquor, 0.5%).

Strain SP 1-3 was cultured for 24 h at 45°C anaerobically by using a medium consisted of glucose1.5%, rice bran 0.5%, Na-acetate 0.2%, $MgSO_4.7H_2O$ 0.02%, $MnSO_4.4H_2O$ 0.01%, pH 6.5 and added additive described in the table.

in spray-dried granule was calculated to be decreased by 15.09%. It elucidated that this strain was very stable against heating and showed high %survival by spray-dry treatment. The changing of LAB survival in this granule was tested at 4°C and room temperature (~26-30°C) as shown in Table 4. In storing at 4°C or 30°C for 28 days, the cell count of spray-dried granule was maintained more than 10^8 cfu/g. Except the case of sample as a final product left in the nozzle chamber storing at 30°C, the cell concentration decreased to 10^4 cfu/g after 28 days.

4. Laboratory-scale Napier grass silage with the spray-dried strain SP 1-3

Napier grass silage inoculated with the spray-dried granule of strain SP 1-3 was prepared in plastic pouch. Silage was prepared with 2% glucose and kept at 30°C. After opening pouch, the moisture content, silage pH and microbial counts in silage were examined as shown in Table 5. Initial moisture content of material was about 82.5%. The moisture content changed within 70.4-73.9% during ensiling. The pH of silage inoculated with LAB (LAB treatment) was reduced from 4.5 to 3.8 during fermentation, while the pH of silage without inoculation (Control treatment, C) was almost constant (about pH 4.7~4.8). The yeast and enterobacteria cell counts in control treatment were progressively increased, while in the LAB treatment, those counts were repressed at the lower level. During silage fermentation, the cell counts of Clostridia were no-detectable level in both of control silage and strain SP 1-3 inoculated silage.

5. Preparative spray-dried strain SP 1-3 culture solution, named PBS-SP, for Practical-use Napier grass silage

For a better quality silage making, the addition of 1 g granule to 1 kg silage material (fresh matter: FM) is required because it is well known that the safety level of LAB presence on silage material is at least 10^5 cfu/g FM (Ohmomo *et al*, 2002b). Consequently, it is necessary to prepare about 600 g of granule to make 600 kg of silage (30 kg × 20 rolls). However, 600 g may cost a lot and be hard for farmer. For that reason, an easy and convenient method to increase cell mass in granule (silage inoculant) was tried by using a 5-liter plastic bottle of drinking water from the market mixed with optimized medium composition mensioned above to prepare 4.5 litres LAB culture and called as plastic bottle fermentation of spray-dried strain SP 1-3 culture solution (PBS-SP culture). Changing of pH and LAB cell count in a plastic bottle was shown in Fig 1. During 24-h incubation, cell counts (4.45×10^6 cfu/ml) in drinking water increased to 3.85×10^9 cfu/ml and pH of drinking water was reduced from 5.75 to 3.72. This culture broth (about 4.5 L) can cover the addition of silage inoculant for about 4.5 ton of silage material (FM).

Table 4 Changing of the survival cells in spray dried granule of strain SP 1-3.

Stored time (day)	Cell counts in storing at 4°C (cfu/g)		Cell counts in storing at 28-30°C (cfu/g)	
	DSC[1]	SFP[2]	DSC[1]	SFP[2]
1	ND	ND	6.08×10^8	1.87×10^9
7	7.03×10^8	1.68×10^9	7.22×10^8	1.62×10^9
14	6.77×10^8	1.52×10^9	5.72×10^8	7.10×10^7
21	6.25×10^8	1.28×10^9	3.15×10^8	4.84×10^5
28	5.83×10^8	1.12×10^9	1.68×10^8	5.01×10^4

1 DSC: direct sampling from chamber, 2SFP: sampling from product nozzle. ND, not determined. Sample powder was packed in a plastic pouch, sealed by a vacuum sealer and kept at 4°C or ~28-30°C.

Table 5 Changing of microbial cell counts and pH in Napier grass silage inoculated spray dried cell of strain SP 1-3.

Storing time (day)	pH		Cell counts in C1			Cell counts in LAB2		
	C	LAB	LAB	YM	Entero	LAB	YM	Entero
1	4.81	4.50	2.05×10^7	1.37×103	1.40×10^6	2.05×10^7	8.85×10^3	1.40×10^6
7	4.67	3.82	5.72×10^6	1.28×10^5	4.33×10^6	1.73×10^7	6.10×10^4	2.87×10^3
14	4.71	3.78	4.47×10^5	3.22×10^6	4.12×10^4	2.62×10^6	4.05×10^4	1.17×10^3
28	4.72	4.07	4.17×10^4	6.87×10^5	3.66×10^3	4.67×10^5	4.18×10^2	6.37×10^4

1) C: Control (not added), LAB: Added strain SP 1-3; 2)LAB: Lactic acid bacteria, YM: Yeast and Mould, Entero: Enterobacteria.
Silage was prepared in a plastic pouch and kept at room temperature for 28 days (all experiments were triplicate). Inoculum size of strain SP 1-3 was 105 cfu/g.

In addition, the price of additional charge to increase cell mass by the method of PBS-SP culture preparation is calculated as follows: one bottle of 5-liter drinking water in plastic bottle is about 40~45 Baht and medium component mixture for 4.5 liter is about 36~40 Baht. A total cost charge for making 4.5 ton of good quality silage is only 76~85 Baht for one bottle.

6. Practical-use grass silage with PBS-SP culture

Pangola grass silage and Erianthus silage inoculated with PBS-SP culture were prepared in plastic bag. After opening bag which was kept at 30-35°C for 90 days, the silage pH, contents of organic acids, volatile basic nitrogen (VBN) and microbial cell counts were examined as shown in Table 6. Comparing control and LAB treatment, the fermentation quality of silage inoculated with LAB was improved as shown in reducing pH less than 4, increasing lactic acid content of 3.3 and 10.7%, reducing VBN content of 0.012 and 0.2% for Erianthus silage and Pangola grass silage, respectively. These improvements were more remarkable in Erianthus silage. However, the yeast cell count was not reduced by the inoculation of LAB. The high number of yeast cell of 5.7 and 8.6 logCFU/g from Erianthus silage and Pangola grass silage have occurred, respectively. The abundant yeast cell in silage prepared in Thailand was hitherto known (Ohmomo *et al*., 2002a) and this trend was clearly remarkable in the case of Pangola grass silage of this experiment. This abundant yeast cell in silage means the outbreak of aerobic deterioration after opening silo. Actually, the Pangola grass silage pH of 48 h after opening silo raised to almost neutral while the pH was about 3.5 at just open the silo.

In addition, the palatability of silage inoculated with LAB was compared with the control treatment by the cafeteria method (Abe, 2001) using Brahman steers and native Thai steers. However, no significant difference of the intake

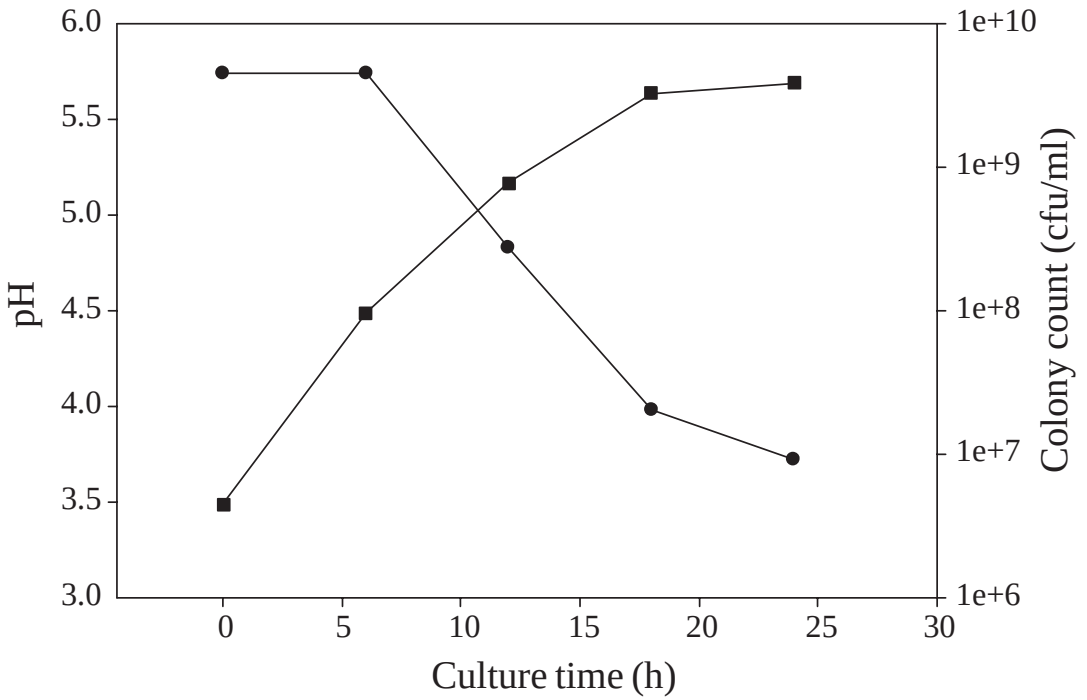

Figure 1 Changes of pH and cell counts of PBS-SP culture. pH, ● , Cell count, ■ . Nutrient power (130 g) described in the text was dissolved in 4.5-litre plastic bottled drinking water, spray-dried granule (200 mg) was suspended into the liquid and kept for 24 h at room temperature. Small amount of culture broth was sampling for pH and cell count analysis for 6, 12, 18 and 24 h.

amount between the control and LAB treatment silages was made sure (data no shown).

CONCLUSION

For the large scale culture of a strain SP 1-3, an optimized medium composed with molasses (2.0%), rice bran (0.5%), yeast extract (0.2%), Na-acetate (0.2%), small amounts of mineral (Mg^{++}, Mn^{++}), pH 6.5 was decided. These components were selected according to stable production and low-cost materials in Thailand. Dried living cells of a strain SP 1-3 (granule) was prepared by spray-dry method. The survival was about 15% and the living cell counts after storing at both of 4°C and 30°C for 28 days were maintained the level of more than 10^8 cfu/g.

For the practical use of this granule at farmer's field, LAB in the form of PBS-SP culture with high concentration of cell mass was prepared by using a 5-liter plastic bottled drinking water from the market. Pangola grass silage and Erianthus silage inoculated with PBS-SP were prepared in plastic bag (each bag was about 20 kg). By the inoculation of PBS-SP culture, the fermentation quality was improved, especially in Pangola grass silage. However, the reduction of yeast cell counts in silage and the palatability were not improved, unexpectedly.

Table 6 Fermentation quality of practical-use silage inoculated LAB strain SP 1-3.

Material	LAB	Silage pH[1]		Organic acid contents[2]			VBN[3]	Cell counts[4]	
		0 h	48 h	L	A	B		LAB	Yeast
Pangola grass	-	3.49	6.78	9.7±0.4	0	1.8±0.5	0.031	6.2±0.2	8.6±0.1
	+	3.51	6.38	10.7±1.1	0	3.3±0.8	0.020	6.3±0.2	8.6±0.1
Erianthus	-	4.40	5.21	1.8±0.7	0.7±0.8	0.2±0.2	0.021	6.8±0.2	5.8±0.2
	+	4.06	4.96	3.3±0.4	1.2±0.2	0.6±0.3	0.012	6.3±0.2	5.7±0.3

[1]pH value at silo opened and after 48 hr; [2]L: lactic acid, A: acetic acid, B: n-butyric acid; [3]VBN: volatile basic nitrogen; [4]LAB: lactic acid bacteria.
Silage was prepared in a plastic pouch and kept at room temperature for 28 days (all experiments were triplicate). Inoculum size of strain SP 1-3 was 10^5 cfu/g.

LITERATURE CITED

Abe, A. 2001. **Measurement for forage intake by animals**, pp 566-569. *In* I. K. Yokendo (ed.). Method for animal nutrition. Tokyo.

Cai, Y. 1999. Chemical analysis for silage, pp 36-42. *In* **the Association for Grassland, Plant Seeds and Animal Production (ed.)**. Guide book for forage evaluation. Tokyo.

Kawamoto, Y. 1999. Nutritive characteristics of silage and animal production in tropical and subtropical regions, pp.202-215. *In* S. Uchida (ed.). **Progress in Silage Study**. Dairy Japan. Tokyo.

Ohmomo, S., S. Nitisinprasert and S. Hiranpradit. 2002a. Silage-making and recent trend of dairy farming in Thailand. **JARQ**. 36: 227-234.

Ohmomo, S., O. Tanaka, H. Kitamoto and Y. Cai. 2002b. Silage and microbial performance, old story but new problems. **JARQ**. 36: 59-71.

Ohmomo, S., S. Nitisinprasert, D. Kraykaw, P. Laemkorn, S. Tanomwongwattana and S. Hiranpradit. 2004. Evaluation of lactic acid bacteria isolates for silage fermentation inoculant in Thailand by using a modified pouch method. **JARQ**, 38: 199-208.