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Screening Lactic Acid Bacteria for Improving the Kanom-jeen Process

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ABSTRACT

The research objective was to select lactic acid bacteria (LAB), which had been isolated from rice slurry in the Kanom-jeen process, for use as a starter culture. Samples of fermented rice, sedimented flour and drained wet flour taken from the Kanom-jeen production process produced 330 presumptive isolates of LAB. The isolates produced 287 strains that were gram positive and catalase negative, which could be categorized into four groups by their gas production ability and cell morphology: homofermentative rod (77.35%), homofermentative cocci (11.20%), heterofermentative rod (0.35%), and heterofermentative cocci (11.55%). The largest population, homofermentative rod (221 strains), was chosen to be clustered with SPSS ver.10.0 using starch hydrolysis activity and acid productivity. From the cluster results, P1 and P39 showed the highest acid productivity (0.91 and 0.86%, respectively) with the largest starch hydrolysis zone (1.4 and 1.2 cm, respectively). After testing P1 and P39 with API 50 CHL, both strains were identified as *Lactobacillus plantarum*. To study their fermentation ability and effect on the physical properties of Kanom-jeen samples, both strains were separately used in producing Kanom-jeen compared with the commercial process. Results showed that the fermentation time (the time required for acid titration to reach 0.95% lactic acid or higher) of Kanom-jeen with P1 was the shortest (24 h). However, the tensile properties in Kanom-jeen made with P1, P39 or the commercial process were not significantly different ($p > 0.05$).

Key words: screening, lactic acid bacteria, Kanom-jeen

INTRODUCTION

Thai rice noodle, also called "Kanom-jeen" is made by a traditional lactic acid fermentation of rice starch noodle and is very popular in Thailand. The traditional process for Kanom-jeen production involves: cleaning the broken rice; soaking the rice and leaving it in clean water to ferment at room temperature for 3-4 days; grinding; filtering to get flour paste; sedimenting for one day to get sedimented flour; draining; steaming the mixture until cooked; kneading; pressing through a mould into boiling water; cooling; and arranging in a nest-liked shape (Niyomvit, 1985). The major characteristics of Kanom-jeen were identified as fermented odor, elasticity and softness (Uchimura *et al*., 1988). The quality of these products varies greatly based on

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the quality of the broken rice, the fermentation process and the quality of the water. Natural lactic acid fermentation during soaking and sedimenting of the rice slurry were considered to benefit the processing technology and end-product qualities in terms of flavor, texture properties, safety and sensory quality of product (Sribuathong *et al*., 2002). However, natural fermentation in the traditional Kanom-jeen process cannot be controlled. Different types and the amount of natural flora in each production run caused many problems with the end product, such as uncontrollable qualities, food safety or quality standards (Judvong, 2002). These problems can be solved by using a starter culture instead of a natural culture. The starter culture was considered the most important factor in the Kanom-jeen fermentation process not only due to its effects on final product characteristics, but also because it prevented the growth of undesirable microorganisms (Ray and Daeschel, 1992). It is vitally important to choose the right culture. Boonmee (1989) found homofermentative lactic acid bacteria were important microorganisms in the Kanom-jeen process. This was previously reported by Toyada (1979) and Unchimura *et al*. (1988). Lactic acid bacteria (LAB) cause rice grain to soften and sour by acidification, which converts starch into lactic acid. Thus, the important characteristics used in screening LAB are its ability to hydrolyze starch and to produce lactic acid. The aim of this research was to screen the lactic acid bacteria, found in fermented rice, sedimented flour and drained wet flour, and use them as starter culture to shorten the fermentation time in the Kanom-jeen process.

MATERIALS AND METHODS

Fermented rice sample

Fermented rice, sedimented flour and drained wet flour provided from traditional Kanom-jeen manufacture in Pathum Thani and Chachoengsao provinces were sampled and kept at 4°C for no longer than 24 h before analysis. Experiments were set up in triplicate.

Isolation of lactic acid bacteria (LAB) strains

Samples consisting of 1 g of fermented rice, sedimented flour and drained wet flour were blended and diluted with sterilized 0.1% (w/v) peptone solution. The diluted sample was cultured on de Man Rogosa and Sharpe (MRS) agar (Merck, Germany) containing 0.004% (w/v) bromocresol purple, 1% (w/v) calcium carbonate, 0.01% (w/v) sodium azide and incubated at 35°C for 48 h (Harrigan and McCane, 1976). Bacteria colonies, which changed the color of bromocresol purple to yellow and produced a clear zone around colonies, were counted as tentative colonies of LAB. Each tentative colony of LAB was streaked on MRS plates and incubated at 35°C for 48 h for purification. Then, the isolated colonies were selected and grown in MRS slant with 2% (w/v) calcium carbonate at 35°C for 48 h and stored at 4°C.

Screening of homofermentative rod lactic acid bacteria strain

Each isolate of tentative LAB was activated in MRS slant at 35°C for 48 h and transfered to 10 ml MRS broth at 35°C for 24 h. After that, the tentative LAB culture was subjected to gram strain and morphological observation and tests for catalase reaction and gas production (Harrigan and McCane, 1976). Isolates of tentative LAB that were gram positive and catalase negative were confirmed as LAB and categorized into four groups by gas productivity and cell morphology: two were homofermentative (without gas production) with rod or cocci, and two were heterofermentative (with gas production) with rod or cocci.

Clustering homofermentative rod lactic acid bacteria

Acid productivity

One loop of each homofermentative rod, lactic acid, bacteria strain was inoculated into MRS broth, grown at 35°C for 24 h and centrifuged at 8000 rpm 15 min. One ml of supernatant was collected and then assayed for the acid productivity by a titration method (AOAC, 2000).

Starch hydrolysis activity

Homofermentative rod, lactic acid, bacteria strains were individually pour plated on starch agar for 3 days at 35°C and then iodine was added to check if the starch had hydrolyzed by the appearance of a clear zone (Wistreich and Lechtman, 1980). The diameter of the clear zone was used as a measurement of starch hydrolysis activity.

Cluster analysis

Results of acid productivity and starch hydrolysis activity of homofermentative rod, lactic acid, bacteria strains were clustered in between-group linkages for three groups (>200 samples) using SPSS (SPSS software for Windows release 10.0, SPSS Inc, Chicago, IL).

Isolates with high acid productivity and starch hydrolysis activity were selected for the performance test.

Identification test

Homofermentative rod, lactic acid, bacteria strains that were high in acid productivity and starch hydrolysis activity were confirmed using the API 50 CHL (bioMerieux).

Performance test

Liquid culture preparation

Each selected strain was maintained in MRS slant and grown at 35°C for 48 h. One loop of each isolate was inoculated into MRS broth grown at 35°C for 24 h and centrifuged at 8000 rpm for 15 min. Cells were washed twice in sterilized water and 1 g was transferred into 10 ml of sterilized buffer solution giving a final concentration of 10^{10} CFU/ml.

Fermentation time study

Broken rice provided from traditional Kanom-jeen manufacture in Chachoengsao province was washed twice and then soaked in distilled water with a ratio of rice to water of 1: 2 for 6 h and wet milled to get rice slurry, which was then subjected to further Kanom-jeen processing as shown in Figure 1. Fermentation time of the inoculated Kanom-jeen was studied by sampling 30 g of inoculated rice slurry every 4 h, with pH measured using a pH meter (HI 9025, Germany) and acid titration as lactic acid (AOAC, 2000) until the acid titrate of the inoculated rice slurry reached 0.95% or higher.

Titratable acidity of fermented slurry before steaming

Titratable acidity of rice slurry after fermentation was determined as the percentage of lactic acid (AOAC, 2000). The results were compared among: rice slurry with added selected culture; without adding culture (control); non fermented Kanom-jeen; and commercial Kanom-jeen, which was processed by soaking broken rice for 12 h, wet milling, sedimenting for 24 h and draining for 12 h, instead of following the same process (steps 1-3) shown in Figure 1.

Tensile properties

Tensile properties of Kanom-jeen samples based on the selected strains, control, commercial and non fermented, were measured using a texture analyzer (Lloyd Instrument Model TA 500, USA), with 5 cm distance between the upper and lower sample points and a speed of 3 mm/sec. Stress at max load (Pa), % elongation, work to max load (mJ) and Young's modulus (MPa) were reported using Nexygen Version 3.0 (Lloyd, USA). Tensile properties were determined as stress and work at maximum load, which indicated toughness. Young's modulus indicated

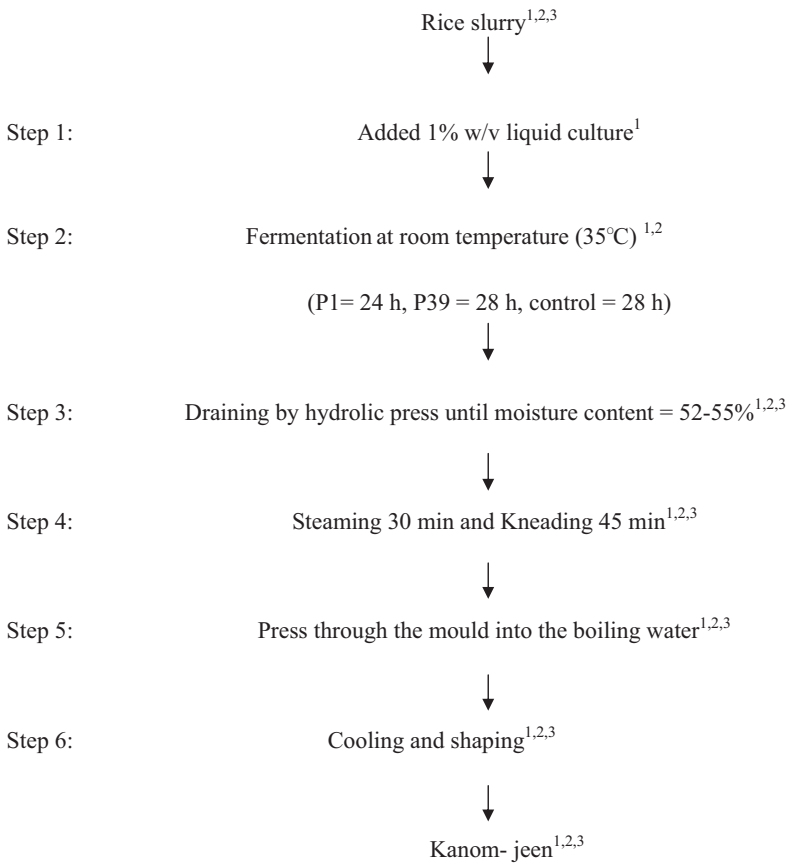

Figure 1 The Kanom-Jeen process.
1/ [1] Process of P1 and P39
2/ [2] Process of control
3/ [3] Process of non fermented

elasticity and elongation indicated stickiness. Each measurement was repeated five times and compared with the commercial Kanom-jeen samples. Statistical analysis was carried out using the SPSS statistics program (version 10.0) for Windows (SPSS Inc, Chicago, IL), using a one-way analysis of variance. Mean comparisons were carried out using Duncan's multiple range test.

RESULTS AND DISCUSSION

Isolation of lactic acid bacteria (LAB) strains

The total number of tentative LAB isolates from fermented rice, sedimented flour and drained wet flour provided by traditional Kanom-jeen manufacture in Patum Thani and Chachoengsao provinces was 163 and 167 isolates, respectively (Table 1).

After testing gram strain and catalase reaction, 287 isolates were tentatively identified as LAB, which were gram positive and catalase negative. They were separated by gas productivity and cell morphology as shown in Table 2.

Homofermentative rods had the largest population of all samples from both sources. The results agreed with other studies (Toyoda, 1979). Fernandez Dies (1983) reported on a fermentation process that used the same raw material, finding

Table 1 The number of tentative lactic acid bacteria isolates from fermented Kanom-jeen process.

Sample	Manufacture	
	Patum Thani	Chachoengsao
Fermented rice	58	59
Sedimented flour	54	51
Drained wet flour	51	57
Total	163	167

Table 2 Ratio of homofermentative rod, homofermentative cooci, heterofermentative rod and heterofermentative cocci LAB from manufacture in Pathum Thani and Chachoengsao provinces.

Sample	Ratio of the culture							
	Homo-rod		Homo-cocci		Hetero-rod		Hetero-cocci	
	P	C	P	C	P	C	P	C
Fermented	37	39	6	8	0	1	7	3
rice	(74.00%)	(76.47%)	(12.00%)	(15.68%)	(0%)	(1.96%)	(14.00%)	(5.88%)
Sedimented	35	36	8	2	0	0	3	7
starch	(74.46%)	(80.00%)	(17.02%)	(4.44%)	(0%)	(0%)	(6.38%)	(15.56%)
Drained	34	40	2	6	0	0	8	5
wet starch	(77.28%)	(78.43%)	(4.65%)	(11.76%)	(0%)	(0%)	(18.60%)	(9.80%)
Total	106	115	16	16	0	1	18	15
	(75.26%)	(78.20%)	(11.36%)	(10.88%)	(0%)	(0.68%)	(12.78%)	(10.20%)
Mean	77.35%		11.20%		0.35%		11.55%	

1/ P = manufacture in Pathum Thani province

2/ C = manufacture in Chachoengsao province

it also had a similar type of natural fermentation. In this case, both sources of traditional Kanom-jeen used broken rice as a raw material.

Cluster analysis

The results of cluster analysis for all homofermentative rod, lactic acid, bacteria strains using acid productivity and starch hydrolysis activity were shown in Figure 2. Only two strains, P1 and P39, were in the group with high acid productivity and starch hydrolysis activity. The starch hydrolysis zone of P1 and P39 was 1.4 and 1.2 cm with 0.91% and 0.86% of acid production, respectively. Sribuathong *et al*. (2002) indicated that acid productivity and starch hydrolysis were important properties in the selection of LAB as a Kanom-jeen starter because the acid from fermentation was the main factor in producing good characteristics in Khanom-jeen. The high acid productivity and starch hydrolysis group of LAB isolated in the study by Sribuathong *et al*. (2002) were in the range of 0.7-1.5% lactic acid with a 0.8-1.0 cm starch hydrolyzed zone. P1 and P39 from this study showed higher starch hydrolysis activity with similar acid productivity compared to LAB isolated in the study reported by Sribuathong *et al*. (2002). However, the larger starch hydrolyzed zone should indicate higher acid productivity, because enzyme glycosidase in microbial cells has been reported to be able to hydrolyze starch into glucose units and convert glucose to lactic acid by the Embden-Meyerhof pathway (Mike and Azam-ali, 1998). Tentative LAB isolates in the high acid

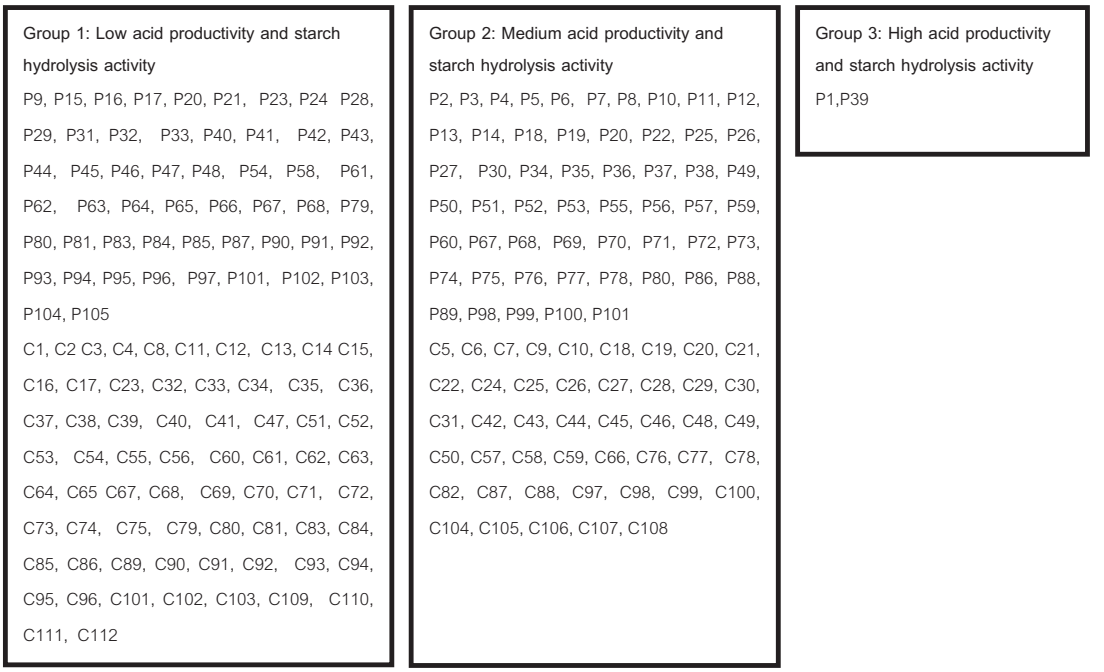

Figure 2 Cluster analysis of homofermentative rod, lactic acid, bacteria strains using acid productivity and starch hydrolysis activity.

productivity and the high starch hydrolysis activity group, P1 and P39, were selected for the next experiment.

Identification test

P1 and P39 were tested, based on their ability to ferment 49 types of carbohydrate using API 50 CHL (bioMerieux), as *Lactobacillus plantarum*. The results of their biochemical profiles are shown in Table 3.

Performance test

Study of fermentation time

Results of acid titratation and the pH value of inoculated flour sampled every 4 h are shown in Figure 3**.** Acidity tended to increase with increasing fermentation time and decreasing pH value. However, Niyomvit (1985) suggested that the optimum percentage of lactic acid and the pH value of fermented rice slurry in Kanom-jeen should be 0.95-1.10% and 3.00-3.50, respectively. From these criteria, the suitable fermentation time of P1 should be 24 h, yielding 1.02% lactic acid and a pH of 3.31 and that of P39 should be 28 h, yielding 1.10% lactic acid and a pH of 3.28.

Titratable acidity of rice slurry and tensile properties of Kanom-Jeen

The control in this study was rice slurry left at room temperature for 28 h without adding liquid culture. The commercial Kanom-jeen was produced by soaking broken rice for 12 h, wet milling, sedimenting for 24 h and draining for 12 h. The titratable acidity of rice slurry samples before steaming are shown in Table 4.

The titratable acidity of fermented rice slurry with P1(24 h), P39(28 h) and the commercial preparation (48h) were not significantly different ($p > 0.05$), but they were significantly different ($p \leq 0.05$) from those of the control (28h without inoculation) and non fermented samples (0h without inoculation). Results showed that rice slurry without starter addition (control), which was left at room

Table 3 Biochemical characteristics of P1 and P39 testing by API 50 CHL (bioMerieux).

Carbon sources	Reaction of P1	Reaction of P39	Carbon sources	Reaction of P1	Reaction of P39	Carbon sources	Reaction of P1	Reaction of P39
Glycerol	-	-	Mannital	+	+	D-raffinose	+	+
Erythritol	-	-	Sorbital	+	+	Starch	-	-
D-arabinose	-	-	α-methyl-D-mannoside	-	-	Glycogene	-	-
L-arabinose	-	-	α-methyl-D-glucoside	-	-	Xylitol	-	-
Ribose	+	+	N-acetyl-glucosamine	+	+	α-gentiobiose	+	+
D-xylose	-	-	Amygdaline	+	+	D-turanose	-	-
L-xylose	-	-	Arbutine	+	+	D-lyxose	-	-
Adonitol	-	-	Esculine	+	+	D-tagatose	-	-
β-methyl-D-xyloside	-	-	Salicine	+	+	D-fucose	-	-
Galactose	+	+	Cellobiose	+	+	L-fucose	-	-
D-glucose	+	+	Maltose	+	+	D-arabitol	-	-
D-fructose	+	+	Lactose	+	+	L-arabitol	-	-
D-mannose	+	+	Melibiose	+	+	gluconate	-	-
L-sorbose	-	-	Sucrose	+	+	2-keto-gluconate	-	-
Rhamnose	-	-	Trehalose	+	+	5-keto-gluconate	-	-
Dulcitol	-	-	Inuline	-	-			
Inositol	-	-	Melezitose	+	+			

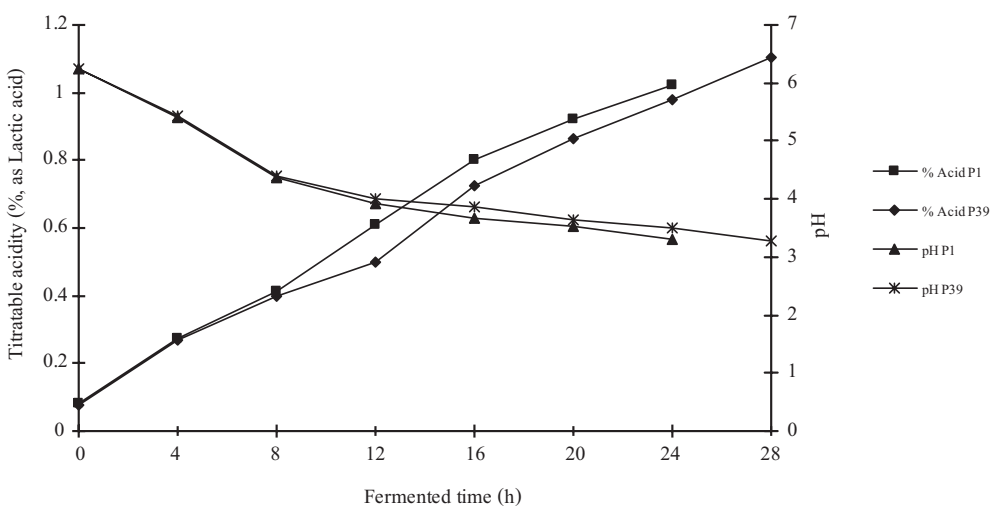

Figure 3 Percentage of lactic acid and pH value in inoculated rice slurry (P1 and P39) at 35°C.

Table 4 Titratable acidity of rice slurry before steaming and tensile properties of Kanom-jeen.

Culture	Titratable acidity (%,as lactic acid)	Tensile properties[1]			
		Stress at max. load (Pa)	% elongation	Work to max. load (mJ)	Young's modulus (Mpa)
Control	0.57 ± 0.05[b]	1.98 ± 0.90[b]	49.44 ± 7.37[b]	2.00 ± 6.01[b]	1.07 ± 2.64[b]
P1	1.03 ± 0.04[a]	2.31 ± 0.94[c]	47.97 ± 8.14[c]	2.80 ± 4.71[a]	1.40 ± 3.94[a]
P39	1.10 ± 0.06[a]	2.30 ± 0.81[c]	48.00 ± 7.44[c]	2.76 ± 5.08[a]	1.38 ± 3.01[a]
Commercial	1.12 ± 0.02[a]	2.34 ± 0.89[c]	47.83 ± 6.13[c]	2.81 ± 3.80[a]	1.46 ± 1.70[a]
Non-fermented	0.08 ± 0.04[c]	0.78 ± 0.98[a]	59.81 ± 9.42[a]	1.17 ± 5.34[c]	0.56 ± 0.81[c]

1/ [1] Mean values are calculated from five sample replicates.
2/ [a-b]Means within the same column with different letters are significantly different (P< 0.05).

temperature (30±1°C) for 28 h, the same fermentation time for P39, did not produce enough acid. With the commercial Kanom-jeen, which also had no starter added, the rice slurry needed to be left at room temperature for at least 48 h in order to have enough acid production in the rice slurry. Thus, the addition of starter culture in rice slurry could reduce the fermentation time in the Kanom-jeen process. Wang *et al*. (2000) showed that increasing the acid content during fermentation caused starch granule degradation, which benefited the texture quality of Kanom-jeen. Results of measurements of the tensile properties of Kanom-jeen in this study are also presented in Table 4. The tensile properties of Kanom-jeen for P1, P39 and the commercial samples were not significantly different ($p > 0.05$), but they were significantly different ($p \leq 0.05$) from the control and non-fermented samples. Kanom-jeen samples that included P1, P39 or the commercial preparation had toughness (indicated by stress at max load and work to max load) and elasticity (indicated by Young's modulus) values that were higher than those of the control and non- fermented samples. However, samples of Kanom-jeen made with P1, P39 or the commercial preparation had less stickiness (indicated by % elongation) than those of the control and non-fermented samples. From this information, fermentation affected the mechanical properties as hydrolyzing the flour changed the composition and structure of flour and caused higher tensile strength (Baik *et al*., 1994). The titratable acidity and tensile properties of Kanom-jeen samples with starter culture or made using the commercial preparation were not significantly different ($p > 0.05$), but the fermentation time of Kanom-jeen with starter culture P1(24 h) was less than that of the commercial (48 h) preparation sample. Therefore, Kanom-jeen with starter culture could shorten the fermentation time.

CONCLUSION

The homofermentative rod group had the largest population (76.68%) among lactic acid bacteria in the samples. Cluster analysis, using acid productivity and starch hydrolysis, indicated that P1 and P39 had the highest acid productivity and the largest starch hydrolysis zone, and were confirmed by the API system as *Lactobacillus plantarum*. From the performance test, P1 and P39 could be used as starter culture to shorten the fermentation time in the Kanom-jeen process.

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