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Screening Lactic Acid Bacteria from Thai Agricultural Products and Wastes for Potential Application on Cassava Starch

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ABSTRACT

An evaluation for high yield and stereospecificity-producing lactic acid bacteria (LAB) isolated from various sources including the waste from starch factories, dairy products, fermented vegetable and meat products was conducted under nourished conditions with various concentrations of sodium lactate (3-20% w/v) and glucose (3-30% w/v) at pH 2 – 8. A total of 156 purified cultures were obtained from 57 samples of which 94 strains were homofermentative, the rest were heterofermentative. A purified strain from fermented pork (DM3 strain) demonstrated the highest stereospecificity of L-type (92.8%). The 16S rDNA gene sequence suggested that this DM3 strain was closely related to *Lactobacillus rhamnosus* with a similarity of 99.94%. DM3 provided a maximum yield (89.6%) and productivity (4.79 g/l.h) when grew under the optimal condition, i.e. pH 6.0, 40^{O}C, an agitation speed of 150 rpm and the initial glucose concentration of 100 g/l. Further study was performed by applying DM3 to produce lactic acid from cassava starch, the least expensive commercially available carbon feedstock in Thailand, using Simultaneous Saccharification and Fermentation (SSF) process. Cassava starch (150-200 g/l) was initially liquefied by α-amylase (0.1% v/w) and subsequently saccharified and fermented, simultaneously, by glucoamylase (0.5% v/w) and DM3 (5% v/v). The yield of lactic acid from DM3 fermentation was 136.8 and 166.2 g/l and the productitivity was 5.70 and 1.39 g/l.h, when the initial glucose concentrations were 150 and 200 g/l, respectively.

Key words: cassava starch, *Lactobacillus rhamnosus*, lactic acid, lactic acid bacteria, simultaneous saccharification and fermentation

INTRODUCTION

Lactic acid (LA) is a versatile industrial chemical used as an acidulant and preservative in food industry, controlled drug delivery system in pharmaceutical industry (Sreenath *et al*., 2001), green solvent and cleaning agents in chemical, moisturizers and skin-lightening agents in cosmetic, a precursor for production of emulsifiers such as stearoyl-2-lactylates for baking industries (Schepers *et al*., 2002), a monomer for biodegradable plastic production and as environmentally-friendly chemicals for various applications such as pH regulators, neutralizers and

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ethyl lactate precursors have urged an expansion of lactic acid market globally. The worldwide lactic acid production has been represented approximately 86,000 tons in 2001 and is expected to reach more than 500,000 tons in 2010 (Bochoux *et al*., 2006).

Lactic acid has been produced commercially either by chemical synthesis or microbial fermentation. The chemical synthesis of lactic acid from petroleum-based feedstock has a substantial disadvantage due to a racemic mixture of L- and D-isomers of lactic acid production which, in some certain applications, cannot be applied economically, unless a costly separation process is employed. In contrast, microbial offers a significant advantage by producing optically pure lactic acid, either L- or D-isomeric type, of which the stereospecificity is strain – dependent. Moreover, microbial fermentation is well-recognized as a green technology, indicating the utilization of renewable biomaterials by an environmentally-friendly process, i.e. fermentation of sugar-based feedstock with lactic acid bacteria. Lactic acid bacteria (LAB) have been identified as the major lactic acid producing strains and most of them are considered as a GRAS (generally regarded as safe) substance (Hofvendahl and Hahn-Hagerdal, 2000). The LAB are facultative anaerobes or microaerophilic ones from the standpoint of oxygen requirement. The LAB are classified as homo- or hetero-fermentative based on product types. Homofermentative strains have produced mainly lactic acid without other metabolic products such as other organic acids, alcohol, aldehyes, ketones and carbon dioxide and are more desirable in industrial uses. The yield of lactic acid from glucose by homofermentative strains generally reaches 90% of the theoretical yield or even greater. Investigation of homofermentative strains with a high production yield is still of interest and the strain selection can be optimized by various concerns principally including the preferred isomeric form of lactic acid, the tolerance to adverse growth conditions, yields and productivities (g/l.h). Indeed, different LAB strains differ in their required growth conditions of which should be optimally undertaken to ensure the most efficiencies of lactic acid production.

In Thailand, there are much diverse sources of lactic acid fermentation being in naturally occurred and commercialized products, that can probably be a source of good-performing LAB such as fermented vegetable, fermented meat products, dairy products and industrial wastes (Christine *et al*., 1999; Noonpakdee *et al*., 2003). The objectives of this study consist of screening of LAB from Thai agricultural and wastes that would be suitable for industrial lactic acid fermentation processes and application of selected LAB strain in producing lactic acid from cassava starch, one of the material abundant and inexpensive carbohydrate feedstock.

MATERIALS AND METHODS

1. Materials

Fermented food products and waste were collected from the commercialized products and the industrial factory (Table 1). Cassava starch was obtained commercially from Sanguan Wongse Industries Co., Ltd. (Thailand). The α-amylase (Liquazyme Supra, Novozymes, Denmark) and glucoamylase (OPTIDEX L-400, Genencor International Inc. USA) employed in this present study were a technical grade. All reagents used were analytical grade (Merck, Germany), unless specified by others.

2. Screening of lactic acid bacteria from various fermented sources

The LAB were isolated from various sources including fermented food products from dairy, vegetable and meat and fermented waste water and solid waste from cassava starch factories, by a serial dilution method using 0.85%

NaCl. The samples were pour plated on MRS agar containing bromocresol purple blue and $CaCO_3$ as an indicator, incubated at 37°C under an anaerobic condition for 48 h. The selected LAB were then cultivated in 5-ml basal medium containing 10 g/l yeast extract, 5 g/l peptone, 0.8 mM $MgSO_4$, 0.3 mM $MnSO_4$, 10 mM sodium acetate, 10 mM KH_2PO_4 and 8.6 mM K_2HPO_4 for 48 h. The culture broth was collected and then assayed for lactic acid concentrations by a titration method. In addition to lactic acid production, the homofermentative identification of purified colonies was carried out using an inverted-tube technique (Tsai *et al.,* 1993). The potential high-yielded and homofermentative strains were selected and subsequently evaluated on their tolerance to high acid (3-20% v/v sodium lactate with pH 2-8) and substrate concentration (3-30% w/v glucose). The strains having potential tolerance to adverse environment, i.e. high lactic acid, low pH and high glucose concentration were then selected for product's stereospecificity identification (L- or D-isomer). For this purpose, the D- and L-lactate concentrations were quantified enzymatically using the test kit of combination "D-lactic acid/L-lactic acid" (Cat. No. 112821m, Boehringer Mannheim, Germany). Subsequently, the strain identification of stereospecific selected strains, cultivated in 10 ml of MRS medium, was performed, based on their abilities to ferment 49 types of carbohydrate, using API 50 CHL test kit (BioMerck, France). During incubation, the carbohydrates were fermented to acids which caused a decrease in pH as detected by a color change of an indicator. The results of their biochemical profiles of each strain were then used for strain identification.

3. Optimal fermentation conditions of selected strains

The fermentation medium contains 10 g/l yeast extract, 5 g/l peptone, 0.8 mM $MgSO_4$, 0.3 mM $MnSO_4$, 10 mM sodium acetate, 10 mM KH_2PO_4 and 8.6 mM K_2HPO_4, which were autoclaved at 121°C for 20 min prior to uses. The batch fermentation of selected strain conducted at 2.5 l working volume in a 5 l fermentor (Model MDL, B.E. Marubishi, Japan) were carried out at various conditions, i.e., pH (5, 6 and 7), temperature (30, 37, 40 and 45°C), the agitation speed (100, 150 and 200 rpm) and initial substrate concentrations (30, 50, 100, 150 and 200 g/l). The seed cultures (5% v/v) were prepared by cultivating in the same fermentation medium at 37°C, 18 h.

4. Application of selected LAB strain in producing lactic acid from cassava starch by Simple Saccharification (SS) and Simultaneous Saccharification and Fermentation (SSF) process

A slurry of cassava starch (30% dry solid) was initially liquefied by α-amylase (0.1% v/w of Liquazyme Supra) in a presence of 40 ppm of Ca^{2+} as a cofactor. The liquefied starch hydrolysates were used as the initial substrate for SS and SSF processes of which the solid contents were adjusted as specified in each experiment.

4.1 Simple Saccharification (SS)

The liquefied starch hydrolysates (100 g/l) were adjusted to the optimum pH at 4-4.5 for further hydrolysis by glucoamylase (0.1% v/w OPTIDEX L-400) at 60°C for 18 h. Then, the obtained glucose syrup was fermented by a selected strain in a batch type using the 5% (v/v) seed cultures prepared by cultivating in the fermentation medium described previously at 37°C, 18 h. The fermentation was accomplished under the optimal condition as determined in a previous study.

4.2 Simultaneous Saccharification and Fermentation (SSF)

The liquefied starch hydrolysates were used as the initial substrate at various concentrations (100, 150 and 200 g/l). In a batch-type mode, glucoamylase enzymes (0.5% v/w

OPTIDEX L-400) were added simultaneously to the samples with the 5% (v/v) seed cultures prepared by cultivating in the fermentation medium described previously at 37°C, 18 h and the fermentation was accomplished at an optimal condition as determined in a previous study (in section 3).

5. Analytical assays

The glucose concentrations in liquefied and glucose syrup were measured by Somogyi-Nelson method (Somogyi, 1952). The density of microbial cells in the medium was monitored by measuring the absorbance at 620 nm using a spectrophotometer (Model Genesys 10 UV: Thermospectronic, USA). The viable cell counts (CFU) were carried out by the dilution spread plate method using MRS agar. The supernatant was removed by centrifugation (at 12,298 g for 10 min) and kept for further determination of lactic acid concentrations by simple titration and chromatographic method.

Total acidity, reported as lactic acid equivalent, was determined by titrating the broth against 0.1 M NaOH. The percentage by weight (% w/w) of acid in the sample was calculated by multiplying the volume of alkali (ml) by the factor of 0.09 (AOAC, 1980). Moreover, the lactic acid concentrations in liquid were accurately investigated by High Performance Liquid Chromatography (HPLC) equipped with an Aminex HPX-87H column (Bio-Rad Laboratories, Inc., USA) at 40°C. The analysis was achieved by using 5 mM H_2SO_4 as a mobile phase with the flow rate of 0.6 ml/min and the Ultraviolet-Visible (UV-Vis) detector (SPD-10AVP, Shimadzu, Japan) was employed.

RESULTS AND DISCUSSION

1. Screening of lactic acid bacteria from various fermented sources

The total plate counts (TPC) of LAB on MRS agar from various sources (Table 1) were varied. The TPC values of waste samples, i.e. cassava pulp and waste water were in the range of $10^6 – 10^7$ CFU/g whereas those of fermented food products were in the wide range of $10^2 – 10^8$ CFU/g. The microbial counts differed among samples due to the different uses of raw material, microflora, source and conditions of microbial growth. From a total of 57 screened samples, 156 strains were isolated of which 94 strains exhibited homofermentative characteristics with a production yield of lactic acid greater than 10 g/l (glucose concentration = 20 g/l, pH 6, at 37 °C for 48 h). To investigate potential strains for industrial uses, the 94 homofermentative and high-yielded strains were evaluated for their fermentation efficiencies under the stress of high lactate concentrations (3, 5, 10, 15 and 20% w/v of sodium lactate) and pH condition (2, 3, 4, 5, 6, 7 and 8). The production yields with various initial glucose concentrations (3, 5, 10, 30% w/v) were also evaluated. All selected homofermentative strains could not grow in the presence of high lactate concentration (at 10% w/v) and only 3 strains did not grow in a media containing 3% (w/v) sodium lactate. A total of 41 strains were able to grow at 5% (w/v) lactate (Table 1). No selected homofermentative strains was able to grow at a very acidic condition (pH =2). However, some strains (n = 42) were capable to grow in an acidic environment (pH = 3) with a low yield. The cultivation of most strains was very efficient at a slightly acidic condition (pH 5-7) with a high yield of lactic acid (12-18 g/l at glucose = 20 g/l). For the effect of initial substrate concentration, there was one strain isolated from fermented pork (being coded as CH6-2) that could not grow when the initial glucose concentration was greater than 20% (w/v). The rest could grow in all evaluated substrate concentrations (3-30% w/v), yielding the lactic acid concentration of 10 – 20 g/l (Table 1).

The selected homofermentative strains

Table 1 Information and some characteristics of lactic acid bacteria screened from various fermented sources.

Sources	No. of samples	No. of purified colonies	Type of fermentation[a]		Fermentation efficiency[b]			Stereospecficity	
			Homo-fermen-tative	Hetero-fermen-tative	Tolerance to lactate[c] (5% w/v)	Tolerance to acid[d] (pH =3)	Tolerance to high substate[e]	L-isomer	D-isomer
Cabbage	6	21	15	3	4	5	3	-	-
Bamboo	3	2	-	2	-	-	-	-	-
Mango	1	3	3	-	-	1	3	-	-
Garlic	1	2	2	-	1	1	-	-	-
Olive	1	1	-	-	-	-	-	-	-
Fermented noodle	1	2	1	1	-	-	-	-	-
Fish	3	8	2	4	1	1	1	-	-
Pork	17	64	49	7	23	22	27	1	-
Mollusk	1	3	1	-	-	1	1	-	-
Crab	2	4	2	1	2	2	2	-	-
Yoghurt	5	8	-	-	-	-	-	-	-
Drinking yoghurt	5	8	3	-	2	1	3	1	-
Cassava waste water	6	17	4	8	4	3	4	-	-
Cassava pulp	5	13	12	1	4	5	9	2	-
Total	**57**	**156**	**94**	**27**	**41**	**42**	**53**	**4**	**0**

[a] All purified colonies were evaluated (n = 156).

[b] Only homofermentative LAB with a production of lactic acid more than 10 g/l were evaluated (n = 94) for further uses.

[c] Tolerance to lactate of LAB was conducted with various sodium lactate concentrations (3, 5, 10, 15 and 20% w/v) at pH 6, temperature 37°C and glucose concentration 20 g/l. No strains could grow at sodium lactate ≥ 10% and 3 strains could not grow at 3%.

[d] Tolerance to acid of LAB was conducted with various pH (2, 3, 4, 5, 6, 7 and 8) at temperature of 37°C and glucose concentration of 20 g/l. No strains could grow at pH = 2, all strains could grow at pH 5, 6, 7 and 8.

[e] The lactic acid production was performed at various glucose concentrations (3, 5, 10, 15, 20, 25 and 30% w/v), at pH 6 and temperature 37°C. 53 strains gave the lactic acid production more than 18 g/l.

with potential tolerance to lactate, acid and high substrate concentrations (n = 53) were subsequently evaluated for their stereospecificity (Table 1). Most strains demonstrated the ability to produce an equivalent quantity of L- and D-isomer. Only 4 strains (DY, DM3, CB(R2)-bf and FCP2) produced lactic acid with highly L-isomer specific (>90%), but none was found to be D-isomer specific. The identification of these highly L-isomer specific strains based on their carbohydrate fermentation capability of different substrates suggested that DY and FCP2 were *Lactobacillus para parcasei* while DM3 and CB(R2)-bf were *Lactobacillus rhamnosus* (Table 2). Among 4 selected strains, DM3 screened from fermented pork is feasible to use for the application trial with cassava starch since it yielded the highest L-specific (92.8%) and the total lactic acid concentration (16.6 g/l). In fact, many studies reported that *L. rhamnosus* is probiotic bacteria (Hilde *et al*., 2005) that can produce exopolysaccharides (Tomasz *et al*., 2003) and lactic acid (Sunhoon *et al*., 2000; Gao *et al*., 2005). A ribosomal DNA-based identification of bacteria by 16S rDNA gene sequencing was used to confirm that the strain DM3 was closely related to *Lactobacillus rhamnosus* with similarities of 99.94%.

Table 2 Summarized characteristics of potential selected strains of lactic acid bacteria screened from various fermented sources with high stereospecificity and acid production.

Selected strain	Source	Type	Lactic acid stereopecificity	Lactic acid concentration* (g/l)
DY	Drinking yoghurt	*Lactobacillus para parcasei*	L-type (89.9%)	16.9
FCP2	Cassava pulp	*Lactobacillus para parcasei*	L-type (91.2%)	13.7
DM3	Fermented pork	*Lactobacillus rhamnosus*	L-type (92.8%)	16.6
CB(R2)-bf	Cassava pulp	*Lactobacillus rhamnosus*	L-type (90.2%)	16.4

* Fermentation was carried out at pH 6 and 37°C with initial glucose concentration of 20 g/l.

2. Optimal fermentation conditions of selected strains

2.1 pH

In order to study the effect of pH, lactic acid fermentation of DM3 was carried out at 37°C with the continuous agitation at 150 rpm. Figure 1a demonstrated the changes in parameters during LAB fermentation without and with controlled pH (at 5, 6 and 7). Without the pH-control system, the cells could be cultivated up to 8 h after inoculation. However, when the pH was dropped to 3, no microbial growth was detected, i.e. the cell density and cell count were constant (cell density = 3.98, cell count = 3.50×10^9 CFU/ml). The optimum pH range for lactic acid production by DM3 was 5 to 7, of which pH 6 gave the shortest time of microbial lag phase and the glucose was completely consumed within 10 h, as compared to 12 h for fermentation at pH 5 and 7. As a result, at pH 6, the highest productivity (2.90 g/l.h) was obtained (Table 3).

Table 3 Effect of pH, temperature and agitation speed on concentration, yield and productivity of lactic acid and glucose residual during fermentation of glucose by a selected strain DM 3 (*Lactobacillus rhamnosus*) screened from Thai fermented pork.

Fermentation conditions		Lactic acid concentration (g/l)	Glucose residual (g/l)	Yield***** (%)	Productivity (g/l.h)
pH*	As is****	13.8	19.3	123.5	0.58
	5	29.5	0.3	98.4	2.46
	6	29.0	0.2	96.7	2.90
	7	29.1	0.2	93.1	2.43
Temperature**	30	29.6	0.2	94.7	2.47
(°C)	37	29.0	0.2	96.7	2.9
	40	29.8	0.2	97.3	3.37
	45	29.1	0.2	97.1	2.43
Agitation	100	28.7	0.4	95.7	2.87
speed***	150	29.8	0.2	97.3	3.73
(rpm)	200	29.0	0.4	92.8	2.90

*Using glucose 30 g/l at temperature = 37°C and the agitation speed = 150 rpm

**Using glucose 30 g/l at pH = 6 and the agitation speed =150 rpm

***Using glucose 30 g/l at pH = 6 and temperature = 40°C

**** without controlling pH

*****Based on the ratio of products produced and substrates used.

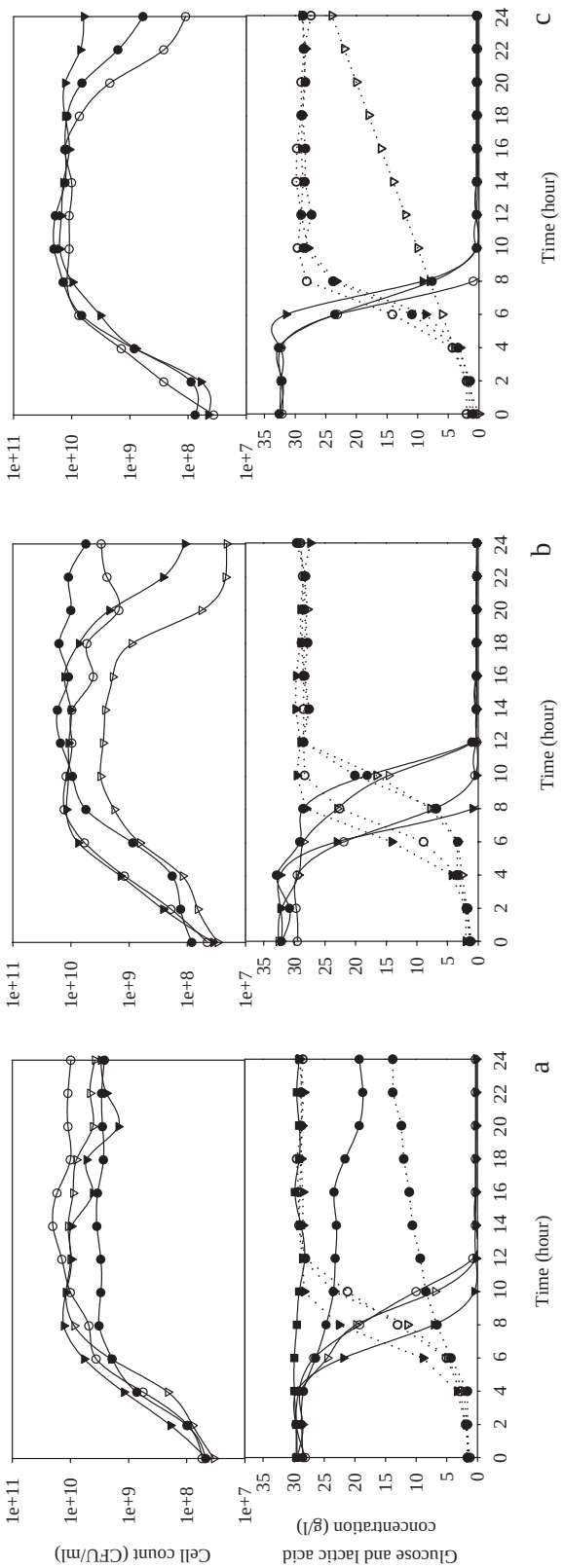

Figure 1 Effect of pH, temperature and agitation speeds on cell counts (CFU/ml), glucose and lactic acid concentrations during fermentation of glucose (30 g/l) by a selected strain DM3 (*Lactobacillus rhamnosus*) screened from Thai fermented pork. a) at various pH including without pH control (●), pH 5 (○), pH 6 (▼) and pH 7 (▽); b) at various temperature including 30°C (●), 37°C (○), 40°C (▼) and 45°C (▽); and c) at various agitation speeds including 100 rpm (●), 150 rpm (○) and 200 rpm (▼).

2.2 Temperature

Figure 1b shows the effect of temperature at 30, 37, 40 and 45°C on lactic acid fermentation by DM3, when the pH was constantly regulated at 6. The optimal temperature was in the range of 30 to 45°C. No significant difference in lactic acid concentrations and yields was observed, indicating a good adaptability of this microbial strain to a wide temperature ranges. At 40°C, the lag phase of cell growth was the shortest (4 h) and the glucose consumption was the fastest when compare with 30 and 37°C. The fermentation periods were 12, 10 and 8 h for 30, 37 and 40°C, respectively. As a result, the fermentation temperature at 40°C provided the highest productivity and yield (3.73 g/l.h and 97.3%), respectively (Table 3).

2.3 Agitation speed

The consequence of agitation speed on lactic acid fermentation efficiency was carried out at a controlled temperature and pH, i.e. 40°C and pH 6. When the agitation speed of 150 rpm was employed, the highest lactic acid concentration, yield and productivity were achieved (29.8 g/l, 97.3% of yield and 3.73 g/l.h, respectively; Figure 1c and Table 3). It could be somehow related to the amount of dissolved oxygen due to stirring of the media.

Different lactic acid bacterial strains differed in their requirement for growth conditions. For the strain DM3, the maximum lactic acid concentrations could be achieved when fermentation was carried out at pH 6, temperature of 40°C and agitation speed of 150 rpm (Table 3), which was in accordance with a previous report (Hofvendahl and Hahn-Hagerdal, 2000) the optimal condition for lactic acid is pH 5-6.8, temperature 30-45°C with continuously agitating at 100-200 rpm. Among these three factors, it was obvious that pH of the medium had the greatest effect on lactic acid production. Goncalves *et al*. (1997) reported a mechanism of lactic acid inhibition on microbial growth and cell metabolism, which related to lactic acid production, is a complex process. The inhibition mechanism could not be simply described exclusively as an effect of lactic acid/lactate equilibrium, nor could it be generalized for a wide rang of extra-cellular pH values. Nevertheless, their studies suggested that the growth inhibition by lactic acid was a function of pH. At high pH, the intracellular dissociated or ionized lactic acid concentration was the inhibitory species whereas, at low pH, the pH effect on bacteria cell growth was, in part, due to the concentrations of non-dissociated forms of lactic acid in the broth. Some researchers had demonstrated that both dissociated and non-dissociated lactic acids had inhibitory effects with the non-dissociated organic acids being more inhibiting than the dissociated one.

2.4 Influence of initial substrate concentration

The effect of the initial substrate concentration was carried out by varying the initial glucose concentrations at 30, 50, 100, 150 and 200 g/l. At a low glucose concentration, the lag phase of microbial cell was shortest and the cell growth was higher, when compared to high glucose concentrations (150 and 200 g/l) (Figure 2). The maximum cell counts were obtained at different fermentation periods; 1.3×10^{10} CFU/ml at 16 h, 1.5×10^{10} CFU/ml at 12 h, 1.5×10^{10} CFU/ml at 12 h, 9.2×10^{9} CFU/ml at 22 h and 5.8×10^{9} CFU/ml at 20 h for the trials with the initial glucose concentration of 30, 50, 100, 150 and 200 g/l, respectively. Furthermore, at the lowest glucose concentration of 30 g/l, microbial cell counts started to decline after 16 h, presumably due to the lack of nutrient, i.e. glucose.

When the initial glucose concentration increased (30-200 g/l), the amount of lactic acid produced also increased, except at 200 g/l (Table 4). In addition, the glucose consumption during fermentation by LAB was completed at

different time intervals, regarding to the initial glucose concentration. The glucose was completely consumed faster at a low initial substrate concentration (the completion periods were 8, 12, 18, and 48 h for the initial glucose concentration of 30, 50, 100 and 150 g/l, respectively). At a very high glucose concentration of 200 g/l, prolonged fermentation time (greater than 120 h) still did not allow a completion of glucose consumption, implying a low production yield (79.2%). The optimal initial glucose concentration for LAB production by DM3 was 150 g/l, relating to the lactic acid concentration in the final medium (130.8 g/l) which was beneficial

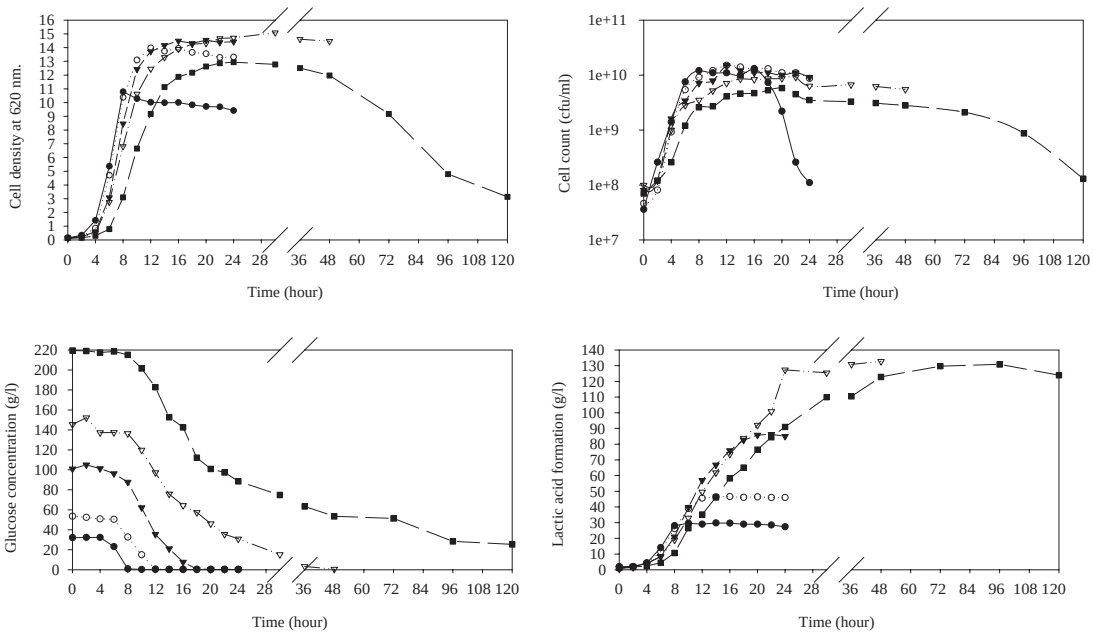

Figure 2 Effect of glucose concentrations on cell density (at 620 nm), cell counts (CFU/ml), glucose and lactic acid concentrations during glucose fermentation by a selected strain DM3 (*Lactobacillus rhamnosus*) screened from Thai fermented pork. The fermentation conditions were at pH 6, temperature 40°C and agitation speed of 150 rpm with various initial glucose concentrations; 30 g/l (●), 50 g/l (❍), 100 g/l (▼), 150 g/l (▽) and 200 g/l (■).

Table 4 Effect of glucose concentrations on concentration, yield and productivity of lactic acid and glucose residual during fermentation* of glucose by a selected strain DM3 (*Lactobacillus rhamnosus*) screened from Thai fermented pork.

Initial glucose concentration (g/l)	Lactic acid concentration (g/l)	Glucose residual (g/l)	Yield** (%)	Productivity (g/l.h)
30	29.8	0.2	97.3	3.73
50	46.6	0.4	89.4	3.88
100	86.2	0.4	89.6	4.79
150	130.8	3.3	92.8	3.63
200	130.8	25.4	79.2	1.09

*The fermentation conditions were at pH 6, temperature 40°C and agitation speed of 150 rpm.

**Based on the ratio of products produced and substrates used.

to subsequent process of purification. However, the highest productivity of lactic acid was achieved when glucose concentration was 100 g/l (4.79 g/l.h) (Table 4).

The results of this work clearly demonstrated that the high initial substrate concentration could have profound effects on cell growths. Evidently, Goncalves *et al*. (1991) reported the kinetics of substrate inhibition on lactic acid production by using glucose concentrations in the range of 50 – 340 g/l. A maximum of 140 g lactic acid / l was reached when 200 g/l of glucose was used, resulting in a 70% yield. The initial substrate concentration may affect the lag phase of cell growth whereas substrate inhibitory effects in lactic acid fermentation may be negligible. Perhaps, the decrease in acid productivity at high initial glucose concentrations could partly attribute to an increased accumulation of lactate in the environment.

3. Application of selected LAB strain in producing lactic acid from cassava starch by Simple Saccharification (SS) and Simultaneous Saccharification and Fermentation (SSF) process

In this study, a comparison on lactic acid production of DM3 using cassava starch hydrolysates between SS and SSF process was investigated at the initial substrate concentration of 100 g/l of starch. In SS process, a high yield (97 %) of lactic acid was obtained in 16 h, exhibiting a high productivity (6.08 g/l.h). Yet the productivity decreased if the saccharification time was also considered (productivity = 4.05 g/l.h). In SSF process, no separate process of saccharification was employed. The liquefied syrup was directly subjected to saccharification by glucoamylase simultaneously with fermentation by LAB. A slight modification in saccharification process, i.e. an increase of enzyme concentration was carried out to optimize and facilitate the microbial growth in SSF process (data not shown). As a result, SSF process could provide comparable fermentation efficiency as indicated by % yield (97 and 92% for SS and SSF process, respectively) (Table 5). A slight reduction of lactic acid productivity by SSF process was evident (5.43 g/l.h), but if the saccharification time in SS process was considered (productivity = 4.05 g/l.h), a productivity of lactic acid could be much improved by SSF process.

The effect of the initial starch concentration in SSF process was further carried out using the initial liquefied starch concentrations of 100, 150 and 200 g/l. The glucose amount was negligible at the initial time of SSF process (< 2% of theoretical total sugar content). The amounts of glucose at the time of inoculation were approximately 6.8 g/l and increased to the maximum of 59.7 g/l at 8 h, 87.5 g/l at 6 h and 136.7 g/l at 6 h for the initial liquefied starch concentration at 100, 150 and 200 g/l, respectively. After that, the glucose concentration slowly declined. Similar to the results obtained by Anuradha *et al*. (1999), our results indicated that there was an initial phase, where the saccharification rate, i.e. glucose production, was greater than the fermentation rate, i.e. glucose consumption, where the glucose was accumulated. Therefore, a lag period in the fermentation was introduced in the simulation. This might be due to the effect of high starch concentrations in the broth. During this period, the cells did not grow, but used glucose for cell maintenance only. Furthermore, initially glucose in the broth accumulated as the fermentation was the rate limiting step. Once the cells adjusted to the medium and started to grow, the saccharification became the rate limiting step and the glucose concentration decreased. The glucose concentration gradually decreased to zero where the saccharification rate was equivalent to the fermentation rate. At this stage, the cells were in the stationary phase and produced lactic acid mainly through maintenance process. With this SSF process of starch, the strain DM3 could

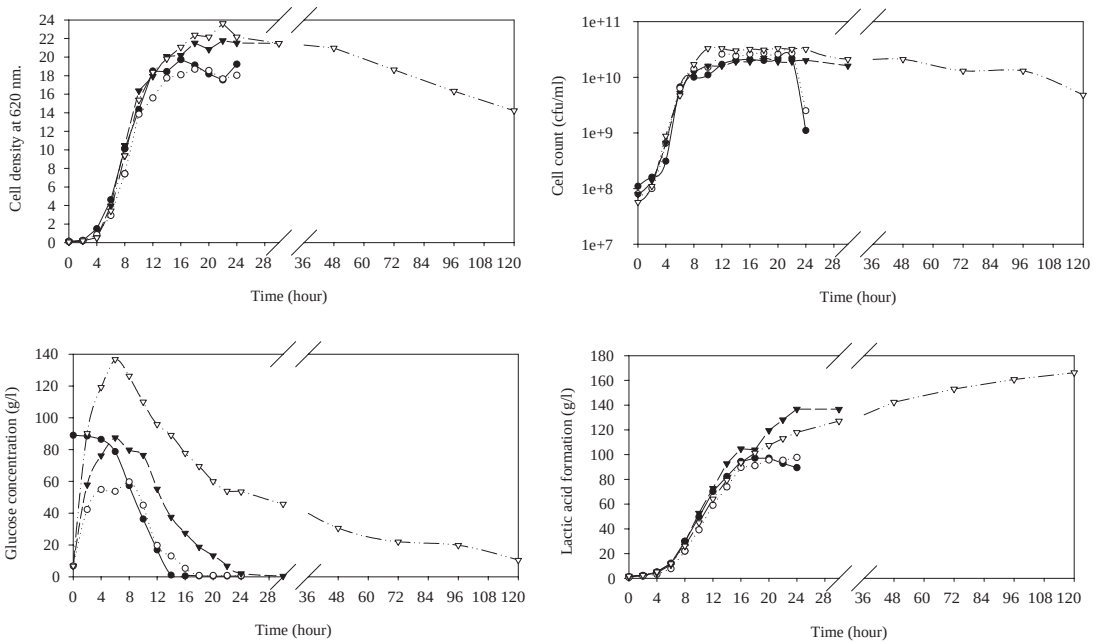

Figure 3 Changes in cell density (at 620 nm), cell counts (CFU/ml), glucose and lactic acid concentrations during fermentation of cassava starch hydrolysates by a selected strain DM3 (*Lactobacillus rhamnosus*) screened from Thai fermented pork using cassava starch as a substrate by Simple Saccharification (SS) and Simultaneous Saccharification and Fermentation (SSF) process at various substrate concentrations; SS 100 g/l (●), SSF 100 g/l (❍), SSF 150 g/l (▼) and SSF 200 g/l (∇).

Table 5 Some parameters during lactic acid fermentation by a selected strain DM3 (*Lactobacillus rhamnosus*) screened from Thai fermented pork using cassava starch as a substrate by Simple Saccharification (SS) and Simultaneous Saccharification and Fermentation (SSF) process at various substrate concentrations**.**

Parameter	SS (100 g/l)	SSF (100 g/l)	SSF (150 g/l)	SSF (200 g/l)
Cell density at 620 nm	19.73	18.69	21.77	23.62
Glucose residual (g/l)	0.5	0.8	2.0	10.5
Starch consumption (g/l)	100	100	148	189.5
Starch consumption (g)	250	250	370	473.8
Substrate utilization (%)	100	100	98.7	94.8
Total lactic acid formation (g/l)	97.2	97.7	136.8	166.2
Total lactic acid formation (g)	243.0	230.0	342.0	390.6
$\%Yield_{P/S}$	97	92	92	82
Fermentation time (h)	16	18	24	120
Productivity(g/l.h)	6.08(4.05*)	5.43	5.70	1.39

* Using a total time of fermentation and Saccharification (24 h).

overcome the limitation of high initial substrate concentrations which had profoundly affected cell growths, as evidenced by the higher cell growths, when compared to fermentation of glucose (> 10 folds). SSF, therefore, has an advantage that the cells are capable to be subjected to the environment with a high substrate concentration, but less glucose accumulation, leading to a lower cell growth inhibition. This benefit was more pronounced when a high substrate concentration, i.e. 200 g/l was used (the maximum lactic acid concentrations was 166.2 and 130.8 g/l when SSF and SS process were compared, respectively, which were approximately 1.27-folds greater).

CONCLUSION

The lactic acid production was operated successfully when commercial cassava starch was used. To decide the most practical process for industrial purposes, the optimal fermentation conditions (i.e. high percentage of yield and/or high lactic acid concentration and/or high productivity) are not always the most preferred from an economical point of view. In fact, the cost of substrate and downstream processing are proportionally high and must be also taken into consideration. The substrate is usually an issue of geographic availability. If the substrate is expensive, the yield should be maximized, whereas the productivity is maximized if investment costs are high. This work had explored the potential use of selected LAB strain and fermentation condition on an inexpensive resource like cassava starch that can be used further to develop lactic acid production industrially.

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