Malaysian Journal of Microbiology, Vol 13(2) June 2017, pp. 100-108

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Identification and lactic acid production of bacteria isolated from soils and tree barks

Vasana Tolieng[1], Budsabathip Prasirtsak[1], Jaruwan Sitdhipol[2], Nuttha Thongchul[1]and Somboon Tanasupawat[3*]

[1]Institute of Biotechnology and Genetic Engineering, Chulalongkorn University, Bangkok 10330, Thailand.
[2]Bioscience Department, Thailand Institute of Scientific and Technological Research (TISTR), PathumThani 12120, Thailand.
Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand.
E-mail: Somboon.T@chula.ac.th

Received 8 August 2016; Received in revised form 7 October 2016; Accepted 13 October 2016

ABSTRACT

Aims: The objective of this research was to isolate and identify lactic acid producing bacteria from soils and tree barks in Thailand. Their acid production was also determined.
Methodology and results: Eleven bacterial strains isolated from soils and tree barks were screened for their lactic acid production. They were divided into 4 groups based on their phenotypic characteristics and 16S rRNA gene sequence analyses. Group I (3 isolates) identified as *Lactococcus* produced L-lactic acid ranged from 72.32 ± 0.707-77.47 ± 0.184 g/L, yield of 0.96 ± 0.011-1.06 ± 0.008 g/g, productivity of 1.00 ± 0.010-1.08 ± 0.003 g/L.h and optical purity was 100%. Group II (3 isolates) identified as *Enterococcus hirae*, produced L-lactic acid ranged from 31.56 ± 0.424-34.86 ± 0.283 g/L, yield of 1.06 ± 0.008-1.23 ± 0.036 g/g, productivity of 0.44 ± 0.006-0.48 ± 0.004 g/L.h and optical purity was 98.6-100%. Group III (3 isolates) identified as *Bacillus coagulans*, produced L-lactic acid ranged from 48.48 ± 0.283-93.51 ± 0.552 g/L, yield of 1.00 ± 0.001-1.07 ± 0.005 g/g, productivity of 0.67 ± 0.004-1.30 ± 0.008 g/L.h and high optical purity of the tested isolate was 99.56%. Group IV (2 isolates) identified as *Sporoactobacillus*. Only NN2 produced D-lactic acid concentration 87.64 ± 0.375 g/L, yield of 0.83 ± 0.000 g/g, productivity of 1.22 ± 0.005 g/L.h and optical purity was 96.16%. All isolates produced lactic acid when 120 g/L of the initial glucose was used as a substrate.
Conclusion, significance and impact of study: Lactic acid producing bacteria are isolated from soils and tree barks. The coccal isolates could produce high L-lactic acid concentration with high optical purity while the spore-forming isolates produces L- and D-lactic acid which are useful for food preservation, chemical in pharmaceutical, cosmetic and textile industries.

Keywords: *Bacillus*, *Enterococcus*, *Lactococcus*, *Sporolactobacillus*, lactic acid

INTRODUCTION

Lactic acid (2-hydroxypropionic acid or $CH_3CHOHCOOH$) can be produced via chemical synthesis or microbial fermentation. Chemical synthesis requires petroleum feedstocks and yields the racemic mixtures of lactic acid while microbial fermentation utilizes the renewable feedstocks which low cost substrate to produce an optically pure L-or D-lactic acid depending on the organism under mild conditions resulting in low energy consumption. Lactic acid bacteria present their ability to produce D, L, and DL-lactic acid, depending on their lactate dehydrogenases and racemases. It had been reported that L-lactic acid was produced in *Enterococcus* species, *Lactobacillus* species, *B. coagulans*, *B. subtilis* and *Lactococcus* species (Hujanen and Linko, 1996; Michelson *et al*., 2006; Chen *et al*., 2013; Gao and Ho, 2013; Yokaryo and Tokiwa, 2014). Pure isomers of L- or D-lactic acid, are more valuable than the racemic DL form because each isomer has many applications in diverse industries such as an acidulant, flavour enhancer, preservative in the food, cosmetics, pharmaceutical, leather, textile industries and a monomer building block of the biodegradable plastic (Wee *et al*., 2006; John *et al*., 2009; Xu *et al*., 2010; Sobrun *et al*., 2012).

Recently, the current demand of L-lactic acid is increasing due to the increasing trend of replacing petroleum based plastic by biobased plastic derived from plant materials such as polylactic acid (PLA) (Wee *et al.*, 2006). Therefore, this study deals with the isolation and identification of lactic acid producing bacteria that exhibit high lactic acid production and optical purity from soil and

*Corresponding author

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Table 1: Samples, location, isolate number, group, 16SrRNA gene sequence similarity (%) and identification.

Samples	Province	Isolate no.	Group	% Similarity	Identification
Bark of *M. indica* L.	Bangkok	BK12-1	I	100	*L.lactis* subsp. *lactis*
Soil	Mahasarakham	MK29-2	I	99.93	*L. taiwanensis*
Soil	Mahasarakham	MK29-3	I	99.85	*L. taiwanensis*
Soil	Saraburi	SB6-2	II	99.3	*E. hirae*
Bark of *A. crassna* Pierre ex H. Lec.	Bangkok	BK10-1	II	99.93	*E. hirae*
Soil	Bangkok	BK13-1	II	100	*E. hirae*
Bark of *S. saman* (Jacq.) Merr.	Bangkok	BK9-1	III	99.39	*B. coagulans*
Bark of *S. saman* (Jacq.) Merr.	Bangkok	BK9-2	III	99.38	*B. coagulans*
Bark of *S. saman* (Jacq.) Merr.	Bangkok	BK9-3	III	99.48	*B. coagulans*
Soil	Nakhon Nayok	NN2	IV	99.93	*S. laevolacticus*
Soil	Nakhon Nayok	NN3-1	IV	99.99	*S. spathodeae*

tree barks in Thailand.

MATERIALS AND METHODS

Sources and isolation methods

The soils collected from Mahasarakham, Saraburi, Nakhon Nayok, Bangkok provinces, and the bark of trees including *Mangifera indica* L., *Aquilaria crassna* Pierre ex H. Lec., *Samanea saman* (Jacq.) Merr collected from Bangkok, Thailand were used for the bacterial isolation (Table 1). A 0.25 g of sample was enriched in 5 mL Glucose-Yeast extract-Peptone (GYP) broth (Prasirtsak *et al*., 2013) containing (per liter) 10 g glucose, 5 g yeast extract, 5 g peptone, 250 mg KH_2PO_4, 250 mg K_2HPO_4 and 10 mL salt solution (400 mg $MgSO_4 \cdot 7H_2O$, 20 mg $MnSO_4 \cdot 5H_2O$, 20 mg $FeSO_4 \cdot 7H_2O$ and 20 mg NaCl per 100 mL solution) and incubated under anaerobic conditions at 37 °C for 3 days. The isolates were streaked on GYP agar plate containing $CaCO_3$ (0.5%) and incubated at the same temperature until the colonies were developed. They were purified and kept on GYP agar slant for further study.

Identification methods

Phenotypic characterization

Phenotypic characteristics such as morphological and cultural of the isolates were investigated on GYP agar plate containing $CaCO_3$ as acid production indicator, after incubated under anaerobic conditions at 37 °C for 3 days. Gram reaction, spore formation, gas formation, catalase activity, nitrate reduction, hydrolysis of arginine and starch, growth at different temperatures (10-50 °C), at different pH values (4-8.5) and different NaCl concentrations (w/v) were performed as previously described (Tanasupawat *et al*., 1992; Tanasupawat *et al*., 1998). Cell shape and spore formation were observed under the light microscope and scanning electron microscope (JSM-5410LV and JSM-6610LV, Tokyo, Japan). Acid formation from various carbohydrates were tested as previously described (Tanasupawat *et al*., 1998). The isomer of lactic acid was analyzed by using high-performance liquid chromatography (Prasirtsak *et al*., 2013).

Genotypic characterization

The 16SrRNA gene was PCR amplified using primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3'), 518F (5'-CCAGCAGCCGCGGTAATACG-3'), 800R (5'-TACCAGGGTATCTAATCC-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') and the amplified 16SrRNA gene sequence was analyzed by Macrogen®, Korea. The sequences of strain were aligned with selected sequences obtained from GenBank by using CLUSTAL_X version 1.81. The alignment was edited manually to remove gaps and ambiguous nucleotides prior to the construction of phylogenetic trees. A phylogenetic tree was constructed by the neighbor joining method (Saitou and Nei, 1987) with the program MEGA 6 (Tamura *et al*., 2013). The confidence values of individual branches in the phylogenetic tree were determined by using the bootstrap analysis of (Felsenstein, 1985) based on 1000 replications. The values for sequence similarity among the closest strains were determined using the EzTaxon server (Kim *et al*., 2012).

Determination of lactic acid and end product

The bacterial isolates were divided into two groups based on the catalase activity. First group, catalase negative isolates were subcultured on the freshly new GYP agar slant and incubated at 37 °C for 48 h under anaerobic condition. The preculture flask consisting of (per liter 10 g glucose, 5 g yeast extract, 5 g peptone, 0.25 g KH_2PO_4, 0.25 g K_2HPO_4, 5 g $CaCO_3$ and 10 mL salts solution) was incubated at 37 °C under anaerobic condition for 26 h. Later 1 mL preculture broth was inoculated into 49 mL fermentation medium (pH 6.80) consisting of (per liter) 120 g glucose, 20 g yeast extract, 10 g peptone, 0.50 g KH_2PO_4, 0.50 g K_2HPO_4, and 20 mL salts solution. After that 4 g $CaCO_3$ was added into the culture broth. The second group, catalase positive isolates were subcultured on the freshly new GYP agar slant and incubated at 37 °C for 48 h under aerobic condition. The preculture flask consisting of (per liter 10 g glucose, 15 g yeast extract, 4

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

g NH_4Cl, 0.50 g KH_2PO_4, 0.50 g K_2HPO_4, 5 g $CaCO_3$ and 20 mL salts solution) was incubated at 37 °C and shaked/swirled at 200 rpm for 5 h. Later 25 mL preculture broth is transferred into 25 mL glucose solution (240 g/L) containing 4 g $CaCO_3$. They were incubated at 37 °C for 72 h under ambient condition. At the end of fermentation, the supernatant was analyzed of lactic acid and the residual glucose by high-performance liquid chromatography (HPLC; Biorad, Aminex HPX-87H ion exclusion organic acid column, 300 mm × 7.8mm) maintained at 45 °C in a column oven (Shimadzu-CTO-6A). An eluent, 0.005 M H_2SO_4, was pumped through the system at the flow rate of 0.6 mL/min (Shimadzu-LC-10Avp). A refractive index detector (Shimadzu-RID-10A) was used to detect the organic compounds detail.

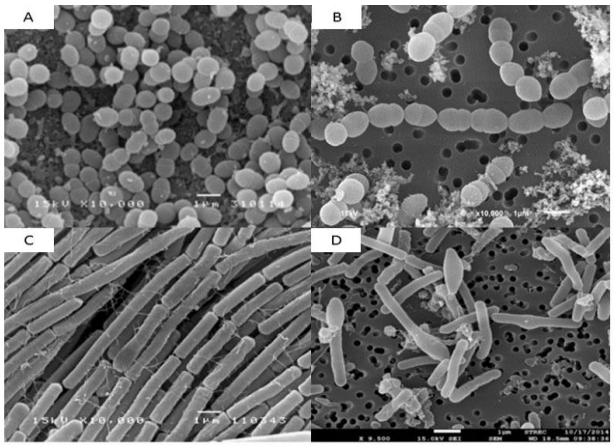

Figure 1: Scanning electron micrograph of isolates BK12-1 (Group I), A; BK13-1 (Group II), B; BK9-1 (Group III), C; and NN2 (Group IV), D on GYP agar plates.

RESULTS AND DISCUSSION

Identification

Six coccal isolates, Gram-positive and non-spore forming bacteria and 5 Gram-positive and spore forming bacteria were characterized. They showed negative reaction to catalase activity (except 3 isolates of *Bacillus*), nitrate reduction and hydrolysis of starch. They were homofermentative lactic acid bacteria with high optical purity of lactic acid from glucose fermentation. All isolates were divided into four groups based on the cell form, spore formation, growth at different temperatures and pH, and NaCl concentrations and acid production from various carbohydrates (Tables 1 and 2) including 16SrRNA gene sequence analyses (Figures 2 and 3). Group I consisted of an isolate BK12-1, MK29-2 and MK29-3. Cells were cocci in chain and non-spore forming (Figure 1). Colonies were smooth, circular, convex, and ivory-white in colour (1.0-1.5 mm in diameter). The isolate

Table 2: Phenotypic characteristics of the isolates.

Characteristics	I	II	III	IV
No. of isolates	3	3	3	2
Cell form	Cocci	Cocci	Rods	Rods
Spore formation	-	-	+	+
Growth temp.(°C)	20-45	15-45	20-50	25-40
Growth in NaCl (%)	5	7	5	7
Growth at pH	4.5-8.5	5.5-8.5	4.5-8.5	4.5-8.5
Catalase activity	-	-	+	-
Nitrate reduction	-	-	-(w1)	-
Starch hydrolysis	-	-	-	-
Arginine hydrolysis	-(+1)	-	-	w (-1)
Acid from:				
L-Arabinose	+	-(+1)	+	+(-1)
D-Cellobiose	+	+	+	+
Dextran	-	-(+1)	-	+
D-Fructose	+	+	+	+
D-Galactose	+	+	+	+(-1)
Inulin	-	-(+1)	-(w1)	+
Lactose	+	+	+(-1)	-(+1)
D-Maltose	+	+	+	-(+1)
D-Mannitol	+	-(+1)	+(-1)	-(+1)
D-Mannose	+	+	+	+
D-Melibiose	-(+1)	+	+(-1)	+
Raffinose	-	-(+1)	-(+1)	+
D-Sorbitol	-	-(+1)	-(+1)	-
L-Sorbose	-	-(+1)	-(+1)	+
Sucrose	+	+	+	+
D-Trehalose	+	+	+	+
D-Xylose	+	-	+	+
Isomer of lactic acid	L	L	L	D

+, positive reaction; -, negative reaction; w, weak reaction. Numbers in parentheses indicate the number of isolates showing the reaction.

grew at 20-45 °C, at pH 4.5-8.5 and in 5% NaCl. Only one isolate BK12-1 could hydrolyze arginine. Acid was produced from L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, lactose D-maltose, D-mannitol, D-mannose, sucrose, D-trehalose and D-xylose but did not produce acid from dextran, inulin, raffinose, sorbitol and L-sorbose. Some isolates were variable on acid production from D-melibiose (Table 2). The 16S rRNA gene sequence of isolate BK12-1 showed 100% similarity to *Lactococcus lactis* subsp.*lactis* JCM 5805^T. Isolates MK29-2 and MK29-3 showed 99.9-100% similarity to *Lactococcus taiwanensis* 0905C15^T (Figure 2). Therefore, isolate BK12-1 was identified as *L. lactis* subsp.*lactis*

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

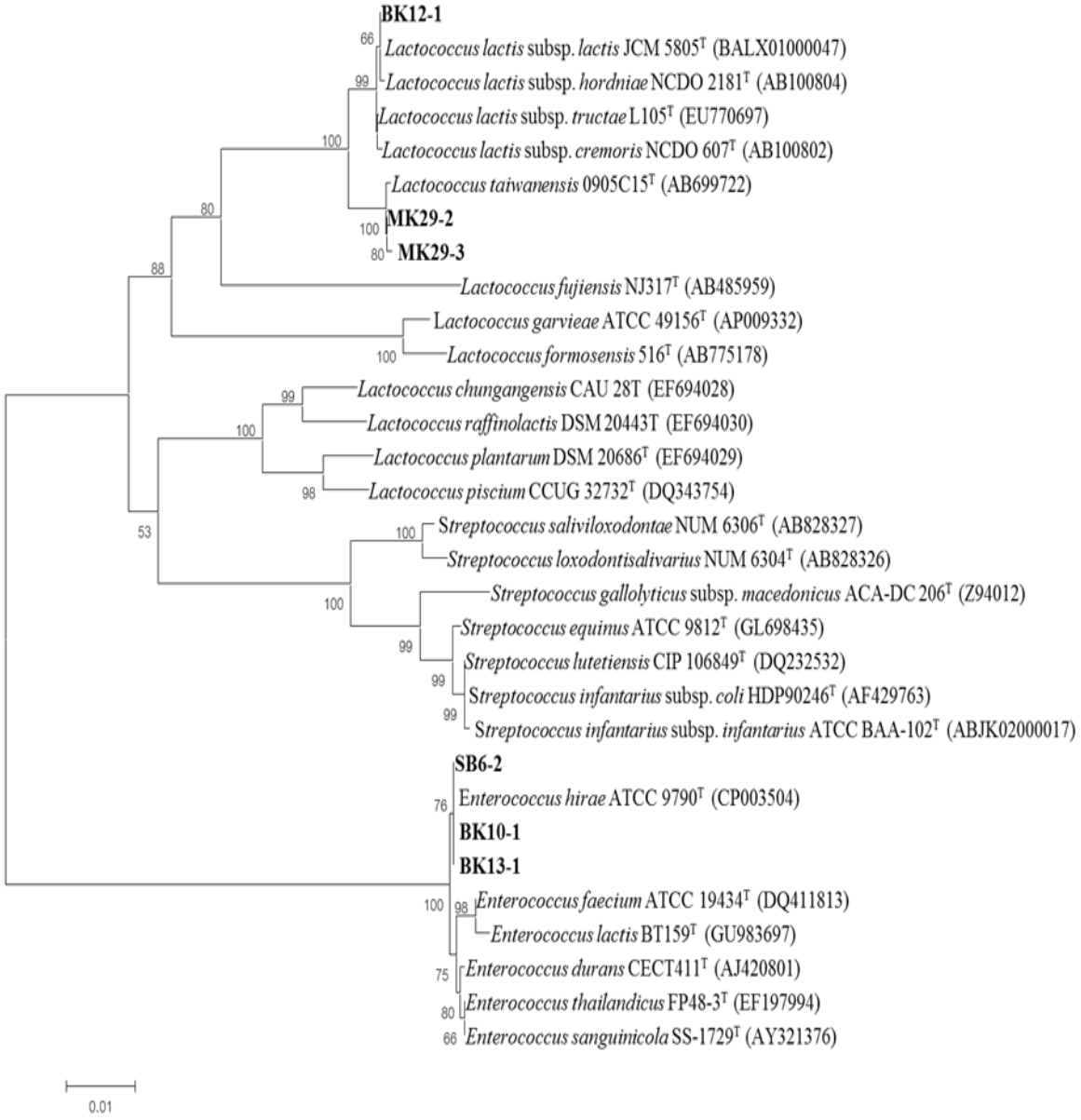

Figure 2: Phylogenetic tree constructed using the neighbor-joining method showing the position of *Lactococcus* and *Enterococcus* isolates and related species based on 16S rRNA gene sequences.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

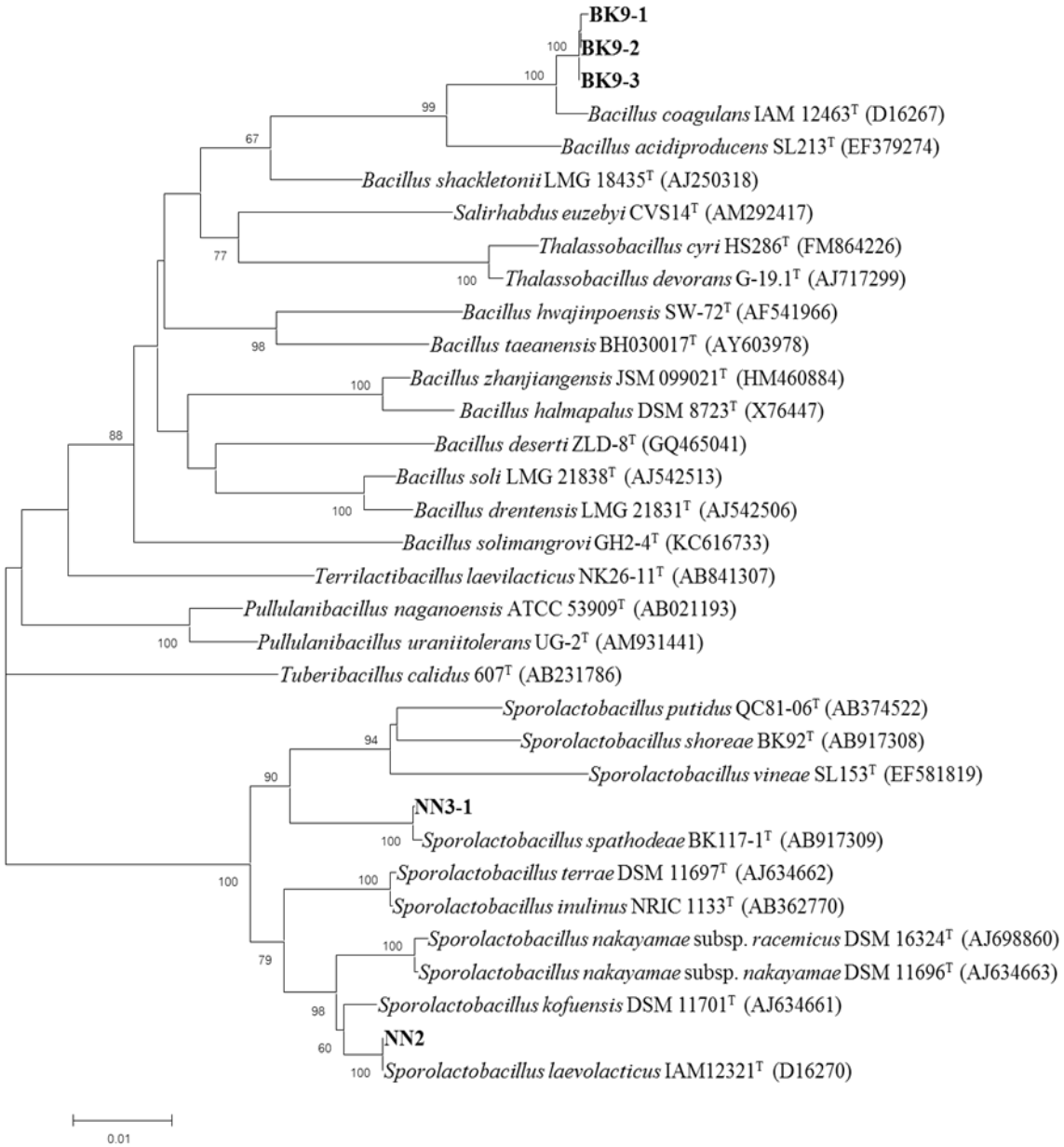

Figure 3: Phylogenetic tree constructed using the neighbor-joining method showing the position of *Bacillus* and *Sporolactobacillus* isolates and related species based on 16S rRNA gene sequences.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

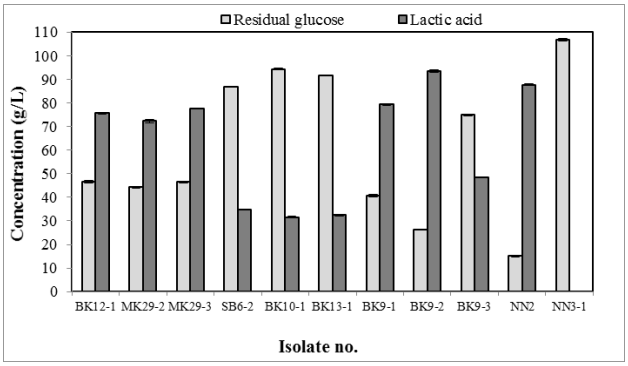

Figure 4: Lactic acid fermentation and residual glucose of isolates.

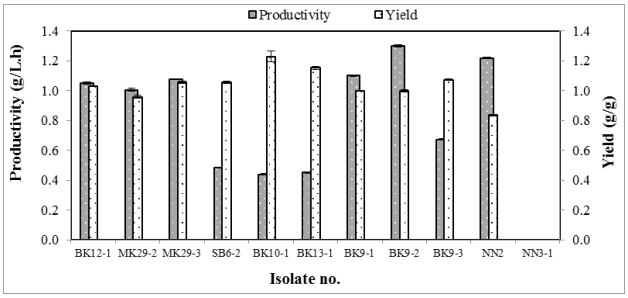

Figure 5: Yields and productivity of lactic acid of isolates cultivated in GYP medium containing 120 g/L glucose at 37 °C, 72 h.

(Fujii *et al*., 2015) while isolates MK29-2 and MK29-3 were *L. taiwanensis* (Chen *et al*., 2013).

Group II consisted of an isolate SB6-2, BK10-1 and BK13-1. Cells were cocci in chains and non-spore forming (Figure 1). Colonies were smooth, circular, convex, and ivory-white in colour (0.4-4.0 mm in diameter). The isolate grew at 15-45 °C, at pH 5.5-8.5 and in 7% NaCl. They showed negative reaction on nitrate reduction and hydrolysis of arginine. Acid was produced from D-cellobiose, D-fructose, D-galactose, D-glucose, D-maltose, D-mannose, D-melibiose, sucrose and D-trehalose. Some isolates produced acid from L-arabinose, dextran, inulin, lactose, D-mannitol, raffinose, sorbitol, and L-sorbose (Table 2). The 16S rRNA gene sequence of these isolates showed 99.3-100% similarity to *E. hirae* ATCC 9790^T (Figure 2). Therefore, they were identified as *E. hirae* (Farrow and Collins, 1985; Devriese *et al*., 2006).

Group III consisted of three isolates, BK9-1, BK9-2 and BK9-3. Cells were spore- forming rods (Figure 1). Colonies were smooth, circular, convex, and ivory-white in colour (1.0-3.1 mm in diameter). These isolates produced catalase, grew at 20-50 °C, at pH 4.5-8.5 and in 5% NaCl. Acid was produced from L- arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, D-maltose, D-mannose, sucrose, D-trehalose and D-xylose but did not produce acid from dextran. Some isolates produced acid from inulin, lactose, D-mannitol, D-melibiose, raffinose, D-sorbitol and L-sorbose. The 16S rRNA gene sequence of isolates showed 99.4-99.5% similarity to *B. coagulans* IAM 12463^T (Figure 3). Therefore, they were identified as *B. coagulans* (Nakamura *et al*., 1988).

Group IV consisted of two isolates, NN2 and NN3-1. Cells were spore-forming rods (Figure 1). Colonies were smooth, circular, convex, and ivory-white in colour (1.3-2.4 mm in diameter). The isolates grew at 25-40 °C, at pH 4.5.0-8.5 and in 7% NaCl. One isolate hydrolysed arginine weakly. Acid was produced from D-cellobiose, dextran, D-fructose, D-glucose, inulin, D-mannose, D-melibiose, raffinose, L-sorbose, sucrose, D-trehalose and D-xylose but one produced acid from L-arabinose, D-galactose, lactose, D-maltose and D-mannitol while all did not produce acid from D-sorbitol (Table 2). The 16S rRNA gene sequence of isolate NN2 showed 99.93% similarity to *Sporolactobacillus laevolacticus* IAM 12321^T (Figure 3). Therefore, it was identified as *S. laevolacticus* (Ludwig *et al*., 2009). The 16S rRNA gene sequence of isolate NN3-1 showed 99.99% similarity to *S. spathodeae* (Figure 3). Therefore, they were identified as *S. laevolacticus* and *S. spathodeae*, respectively (Thamacharoensuk *et al*., 2015).

Lactic acid production

The results on the qualitative isomer of L-and D-lactic acid by using HPLC were shown in Table 3, Figures 4 and 5. *Lactococcus lactis* subsp. *lactis* BK12-1 in Group I which gave final lactic acid concentration 75.67 ± 0.424 g/L, productivity 1.05 ± 0.006 g/L.h, yield 1.03±0.000 g/g and optical purity of L-lactic acid 100% while *L. taiwanensis* MK29-3 produced final lactic acid concentration 77.47 ± 0.184, productivity 1.08 ± 0.003 g/L.h, yield 1.06 ± 0.008 g/g and optical purity of L-lactic acid 100%. *L. lactis* subsp. *lactis* and *L. taiwanensis* strains were reported to isolate from fresh cummingcordia and leaves of sugar cane plant (Cock and de Stouvenel, 2006; Chen *et al*., 2013). A comparison of our strains, *Lc. lactis* IO-1 was reported to produce final lactic acid concentration 7 g/L, productivity 0.11 g/L.h and yield 26% using sugarcane bagasse hydrolysate by batch fermentation (Laopaiboon*et al*., 2010) while *L. lactis* subsp. *lactis* could produce lactic acid from 60 g/L of glucose by batch fermentation, 32 g/L of lactic acid, 63% of yield and 0.67 g/L.h of productivity were obtained (Cock and de Stouvenel, 2006).

Group II, isolates SB6-2, BK10-1 and BK13-1 identified as *E. hirae* produced lactic acid range from 31.56±0.424-34.86 ± 0.283 g/L, yield of 1.06±0.008-1.23 ±0.036 g/g, productivity of 0.44 ± 0.006-0.48 ± 0.004 g/L.h and optical purity was 98.61-100%. It had been reported that *E. hirae* was isolated from raw waste water and tree bark which was obtainedfinal L-lactic acid, productivity and yield were 66.04 g/L, 0.92 g/L.h and 70%, respectively (Prasirtsak *et al*., 2013) while L-lactic acid producing strains were isolated from fermented fish products and elephant faeces in Thailand (Tanasupawat *et al*., 1998; Thamacharoensuk *et al*., 2013).

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Table. 3: Lactic acid and residual glucose of isolates.

Isolate no.	Group	Lactic acid			Initial glucose (g/L)	Residual glucose (g/L)	Optical purity of lactic acid (%ee.)
		Final lactic acid (g/L)	Yield (g/g)	Productivity (g/L.h)			
BK12-1	I	75.67 ± 0.424	1.03±0.000	1.05 ± 0.006	120	46.49 ± 0.537	(L) 100.00
MK29-2	I	72.32 ± 0.707	0.96±0.011	1.00 ± 0.010	120	44.33 ± 0.410	(L) 100. 00
MK29-3	I	77.47 ± 0.184	1.06±0.008	1.08 ± 0.003	120	46.55 ± 0.424	(L) 100.00
SB6-2	II	34.86 ± 0.283	1.06±0.008	0.48 ± 0.004	120	86.92 ± 0.092	(L) 98.61
BK10-1	II	31.56 ± 0.424	1.23±0.036	0.44 ± 0.006	120	94.31 ± 0.417	(L) 100.00
BK13-1	II	32.49 ± 0.354	1.15±0.010	0.45 ± 0.005	120	91.76 ± 0.141	(L) 100.00
BK9-1	III	79.34 ± 0.361	1.00±0.001	1.10 ± 0.005	120	40.70 ± 0.495	(L) 99.56
BK9-2	III	93.51 ± 0.552	1.00±0.007	1.30 ± 0.008	120	26.37 ± 0.092	ND
BK9-3	III	48.48 ± 0.283	1.07±0.005	0.67 ± 0.004	120	74.95 ± 0.354	ND
NN2	IV	87.64 ± 0.375	0.83±0.000	1.22 ± 0.005	120	15.12 ± 0.424	(D) 96.16
NN3-1	IV	50.69 ± 1.021	0.51±0.021	0.70 ± 0.026	80	21.47 ± 0.982	(D) 98.80
NN3-1	IV	14.23 ± 1.382	0.30±0.022	0.20 ± 0.025	100	72.74 ± 0.862	(D) 94.38
NN3-1	IV	0.00 ± 0.000	0.00 ±0. 000	0 ± 0.000	120	106.69 ± 0.559	ND

ND, not determined.

The isolates BK9-1 and BK9-3 in Group III identified as *B. coagulans*, produced final lactic acid range from 79.34 ± 0.361 and 48.48 ± 0.283 g/L and productivity of 0.67 ± 0.004-1.22 ± 0.005, respectively while BK9-2 gave the highest lactic acid of 93.51 ± 0.552 g/L and productivity of 1.30 g/L.h, respectively. *B. coagulans* strains have been isolated from evaporated milk, soils and lignin supplemented minimal medium (Skerman *et al*., 1980; Philip *et al*., 1998; Glaser and Venus, 2014). *B.coagulans* JI12 produced L-lactic acid by fed-batch fermentation with 85 g/L initial xylose and 55 g/L xylose added after 7.5 h, 137.5 g/L and lactic acid yielded 98% and a productivity of 4.4 g/L.h (optical purity >99.5%) (Ye *et al*., 2013) while *B. coagulans* strain IPE22 gave 46.12 g LA from 100 g dry wheat straw with a supplement of 10 g/L corn steep liquid powder at the cellulose (Zhang *et al*., 2014).

The isolates in Group IV identified as *Sporolactobacillus*, only isolate NN2 could produce lactic acid using 120 g/L of glucose while isolate NN3-1 was inhibited by substrate inhibition. NN2 produced D-lactic acid concentration 87.64 ± 0.375 g/L, yield 0.83 ± 0.000 g/g, productivity of 1.22 ± 0.005 g/L.h and optical purity of D-lactic acid 96.16% while NN3-1 produced highest final D-lactic acid 50.69 ± 1.021 g/L, yield 0.51 ± 1.021, productivity 0.70 ± 0.026 g/L.h and optical purity of D-lactic acid 98.8% when 80 g/L of glucose was used. *S. inulinus* strain was reported as the potential D-lactic acid producer with highly optical purity (Fukushima *et al*., 2004) while *S. laevolacticus* DSM442 was reported as an excellent producer for D-lactic acid production (Li *et al*., 2013). In this study, *S. laevolacticus* NN2 and *S. spathodeae* NN3-1 were found to produce D-lactic acid with high optical purity as mentioned above.

CONCLUSION

L-Lactic acid producing bacteria including *L. lactis* subsp. *lactis*, *L. taiwanensis*, *E. hirae* and *B. coagulans* strains were distributed in tree barks and soil whereas spore-forming *S. laevolacticus and S. spathodeae* strains that produced D-lactic acid were isolated from soils. The isolate *B. coagulans* BK9-1 produced L-lactic acid with high optical purity (99.56%) while *S. laevolacticus* NN2 produced D-lactic acid with optical purity 96.16%. These isolates will be the potential bacteria for L-lactic acid or D-lactic acid production in the further study.

ACKNOWLEDGEMENT

This study was supported by the Grant for International Research Integration: Research Pyramid, Ratchadaphiseksomphot Endowment Fund (GCURP_58_01_33_01), Chulalongkorn University.

REFERENCES

Chen, Y. S., Chang, C. H., Pan, S. F., Wang, L. T., Chang, Y. C., Wu, H. C. and Yanagida, F. (2013). *Lactococcus taiwanensis* sp. nov., a lactic acid bacterium isolated from fresh cummingcordia. *International Journal of Systematic and Evolutionary Microbiology* **63, 2405-2409.**

Cock, L. S. and de Stouvenel, A. R. (2006). Lactic acid production by a strain of *Lactococcus lactis* subsp. *lactis* isolated from sugar cane plants. *Electronic Journal of Biotechnology* **9, 40-45.**

Devriese, L., Baele, M. and Butaye, P. (2006). The Genus *Enterococcus*: Taxonomy. *In*: The Prokaryotes: A Handbook on the Biology of Bacteria. Dworkin, M., Falkow, S., Rosenberg,E., Schleifer,K.-H. and Stackebrandt, E. (eds.). Vol. 4. Springer, New York. **pp. 163-174.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Farrow, J. A. E. and Collins, M. D. (1985). *Enterococcus hirae*, a new species that includes amino acid assay strain NCDO 1258 and strains causing growth depression in young chickens. *International Journal of Systematic and Evolutionary Microbiology* **35, 73-75.**

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39, 783-791.**

Fujii, T., Tomita, Y., Ikushima, S., Horie, A. and Fujiwara, D. (2015). Draft genome sequence of *Lactococcus lactis* subsp. *lactis* JCM 5805^T, a strain that induces plasmacytoid dendritic cell activation. *Genome Announcements* **3, e00113-15.**

Fukushima, K., Sogo, K., Miura, S. and Kimura, Y. (2004). Production of D-lactic acid by bacterial fermentation of rice starch. *Macromolecular Bioscience,* **4, 1021-1027.**

Gao, T. and Ho, K. P. (2013). L-lactic acid production by *Bacillus subtilis* MUR1 in continuous culture. *Journal of Biotechnology* **168, 646-651.**

Glaser, R. and Venus, J. (2014). Screening of *Bacillus coagulans* strains in lignin supplemented minimal medium with high throughput turbidity measurements. *Biotechnology Reports* **4, 60-65.**

Hujanen, M. and Linko, Y. Y. (1996). Effect of temperature and various nitrogen sources on L(+)-lactic acid production by *Lactobacillus casei*. *Applied Microbiology and Biotechnology* **45, 307-313.**

John, R. P., Anisha, G.S., Nampoothiri, K. M. and Pandey, A. (2009). Direct lactic acid fermentation: Focus on simultaneous saccharification and lactic acid production. *Biotechnology Advances* **27, 145-152.**

Kim, O. S., Cho, Y. J., Lee, K., Yoon, S. H., Kim, M., Na, H., Park, S. C., Jeon, Y. S., Lee, J. H., Yi, H., Won, S. and Chun, J. (2012). Introducing EzTaxon-e: A prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *International Journal of Systematic and Evolutionary Microbiology* **62, 716-721.**

Laopaiboon, P., Thani, A., Leelavatcharamas, V. and Laopaiboon, L. (2010). Acid hydrolysis of sugarcane bagasse for lactic acid production. *Bioresource Technology* **101, 1036-1043.**

Li, Y., Wang, L., Ju, J., Yu, B. and Ma, Y. (2013). Efficient production of polymer-grade d-lactate by *Sporolactobacillus laevolacticus* DSM442 with agricultural waste cotton seed as the sole nitrogen source. *Bioresource Technology* **142, 186-191.**

Ludwig, W., Schleifer, K. H. and Whitman, W. B. (2009). Genus *Sporolactobacillus*. *In*: Bergey's Manual of Systematic Bacteriology, 2nd. De Vos, P., Garrity, G. M., Jones, D., Krieg, N. R., Ludwig, W., Rainey, F. A., Schleifer, K. H. and Whitman, W. B. (eds.). Vol. 3, Springer, New York. **pp. 386-391.**

Michelson, T., Kask, K., Jõgi, E., Talpsep, E., Suitso, I. and Nurk, A. (2006). L(+)-Lactic acid producer *Bacillus coagulans* SIM-7 DSM 14043 and its comparison with *Lactobacillus delbrueckii* ssp. *lactis* DSM 20073. *Enzyme and Microbial Technology* **39, 861-867.**

Nakamura, L. K., Blumenstock, I. and Claus, D. (1988). Taxonomic study of *Bacillus coagulans* Hammer 1915 with a proposal for *Bacillus smithii* sp. nov. *International Journal of Systematic and Evolutionary Microbiology* **38, 63-73.**

Philip, L., Iyengar, L. and Venkobachar, C. (1998). Cr(VI) reduction by *Bacillus coagulans* isolated from contaminated soils. *Journal of Environmental Engineering* **124, 1165-1170.**

Prasirtsak, B., Tanasupawat, S., Boonsombat, R., Kodama, K. and Thongchul, N. (2013). Characterization of lactic acid producing bacteria from Thai sources. *Journal of Applied Pharmaceutical Science* **3, 033-038.**

Saitou, N. and Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4, 406-425.**

Skerman, V. B. D., McGowan, V. and Sneath, P. H. A. (1980). Approved lists of bacterial names. *International Journal of Systematic Bacteriology* **30, 225-420.**

Sobrun, Y., Bhaw-Luximon, A., Jhurry, D. and Puchooa, D. (2012). Isolation of lactic acid bacteria from sugar cane juice and production of lactic acid from selected improved strains. *Advances in Bioscience and Biotechnology* **3, 398-407.**

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology* and *Evolution* **30, 2725-2729.**

Tanasupawat, S., Ezaki,T., Suzuki, K. I., Okada, S., Komagata, K.and Kozaki, M. (1992). Characterization and identification of *Lactobacillus pentosus* and *Lactobacillus plantarum* strains from fermented foods in Thailand. *Journal of General and Applied Microbiology* **38, 121-134.**

Tanasupawat, S., Okada, S. and Komagata, K. (1998). Lactic acid bacteria found in fermented fish in Thailand. *Journal of General and Applied Microbiology* **44, 193-200.**

Thamacharoensuk, T., Thongchul, N., Taweechotipatr, M., Tolieng, V., Kodama, K. and Tanasupawat, S. (2013). Screening and characterization of lactic acid bacteria from animal faeces for probiotic properties. *Thai Journal of Veterinary Medicine* **43, 541-551.**

Thamacharoensuk, T., Kitahara, M., Ohkuma, M., Thongchul, N. and Tanasupawat, S. (2015). *Sporolactobacillus shoreae* sp. nov. and *Sporolactobacillus spathodeae* sp. nov., two spore-forming lactic acid bacteria isolated from tree barks in Thailand. *International Journal of Systematic and Evolutionary Microbiology* **65, 1220-1226.**

Wee, Y. J., Kim, J. N. and Ryu, H. W. (2006). Biotechnological production of lactic acid and its recent applications. *Food Technology and Biotechnology* **44, 163-172.**

Xu, T. T., Bai, Z. Z., Wang, L. J. and He, B. F. (2010). Breeding of D(-)-lactic acid high producing strain by low-energy ion implantation and preliminary analysis

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

of related metabolism. *Applied Biochemistry and Biotechnology* **160, 314-321.**

Ye, L., Zhou, X., Hudari, M. S., Li, Z. and Wu, J. C. (2013). Highly efficient production of L-lactic acid from xylose by newly isolated *Bacillus coagulans* C106. *Bioresource Technology* **132, 38-44.**

Yokaryo, H. and Tokiwa, Y. (2014). Isolation of alkaliphilic bacteria for production of high optically pure L-(+)-lactic acid. *Journal of General and Applied Microbiology* **60, 270-275.**

Zhang, Y., Chen, X., Luo, J., Qi, B. and Wan, Y. (2014). An efficient process for lactic acid production from wheat straw by a newly isolated *Bacillus coagulans* strain IPE22. *Bioresource Technology* **158, 396-399.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538