Journal of Applied Pharmaceutical Science Vol. 3 (01), pp. 033-038, January, 2013
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2013.30107
ISSN 2231-3354

Characterization of lactic acid producing bacteria from Thai sources

Budsabathip Prasirtsak[a,c], Somboon Tanasupawat[b*], Ruethairat Boonsombat[c], Kentaro Kodama[c] and Nuttha Thongchul[c]
[a]Program in Biotechnology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand.
[b]Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.
[c]Institute of Biotechnology and Genetic Engineering, Chulalongkorn University, Bangkok, Thailand.

ARTICLE INFO

Article history:
Received on: 18/12/2012
Revised on: 02/01/2013
Accepted on: 19/01/2013
Available online: 28/01/2013

Key words:
Lactic acid,
Bacillus, Enterococcus, Lactobacillus, Pediococcus, Sporolactobacillus

ABSTRACT

The aim of this research was to study on the isolation and characterization of lactic acid producing bacteria in Thailand. Ten bacterial strains isolated from soils and plant bark and root were screened for their lactic acid production. They were divided into 7 groups based on their phenotypic and chemotaxonomic characteristics including 16S rRNA gene sequence analyses. Four isolates in Group I were identified as *P. pentosaceus*, and each isolate in Group II to Group VII was identified as *Enterococcus casseliflavus*, *E. hirae*, *Lactobacillus. plantarum*, *L. paraplantarum*, *Bacillus coagulans* and *Sporolactobacillus terrae*, respectively. The isolates in Group I, IV, V and VII produced DL-lactic acid ranged from 54.50-77.31 g/L (45.88-75.11% yield) and 0.76-1.07 g/L.h of productivity. The isolates in Group II, III and VI produced optically pure L (+)- lactic acid ranged from 60.72-89.34 g/L (70.00-74.45% yield) and 0.84-1.24 g/L.h of productivity. *B. coagulans* isolate in Group VI could produce the highest optically pure L-lactic acid (99.75%).

INTRODUCTION

Lactic acid bacteria (LAB) are Gram positive, non-sporing, catalase negative, acid tolerant and produce lactic acid as the major end product from sugar fermentation. LAB consist of bacterial genera such as *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Oenococcus*, *Tetragenococcus*, *Vagococcus*, and *Weissella* (Reddy *et al*., 2008). They were recognized by their ability to produce lactic acid and are commonly distributed in nature such as fermented foods, plants, aminals and soils (Kandler and Weiss, 1986; Tanasupawat and Komagata, 2001; Axelsson, 2004). Lactic acid is an importantly versatile chemical. It has a wide range of applications including the acidulant and preservative in food, the chemical feedstock in pharmaceutical, cosmetic, and textile industries. Lactic acid can be produced via microorganisms by their homofermentation and heterofermentation (Wee *et al*., 2006; Xu *et al*., 2008; John *et al.*, 2009). Recently, the current demand of 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 work deals with isolation and characterization of lactic acid producing bacteria from soils and plant materials in Thailand.

* *Corresponding Author*
Department of Biochemistry and Microbiology,
Faculty of Pharmaceutical Sciences, Chulalongkorn University,
Bangkok 10330, Thailand. Tel: +66-2-2188376.

MATERIALS AND METHODS

Sources and Isolation methods

The soils, plant bark and root collected from Bangkok, Nakhonpathom and Udonthani provinces, Thailand were used for the bacterial isolation (Table 1). A 0.25 gram of sample was enriched in 5 ml GYP broth 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 $MgSO_4.7H_2O$, 20 mg $MnSO_4.5H_2O$, 20 mg $FeSO_4.7H_2O$ and 20 mg NaCl per 10 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 developed. They were purified and kept on GYP agar slant for further study.

Table. 1: Samples, location, isolate number, group and identification.

Sample	Province	Isolate no.	Group	Identification
Soil	Bangkok	CU37-12	I	*P. pentosaceus*
Soil	Bangkok	CU37-21	I	*P. pentosaceus*
Bark of *Tamarindus indica* Linn.	Bangkok	BK78-1	I	*P. pentosaceus*
Root of *Peperomia pellucida*	Nakhonpathom	NK121	I	*P. pentosaceus*
Bark of *Lagerstroemia floribunda* Jack ex Blume.	Bangkok	CU72-2	II	*E. casseliflavus*
Bark of *Samanea saman*	Bangkok	CU39-21	III	*E. hirae*
Bark of *Muntingla calabura*	Udonthani	UD57	IV	*L. plantarum*
Bark of *Muntingla calabura*	Udonthani	UD58	V	*L. paraplantarum*
Bark of *Lagerstroemia floribunda* Jack ex Blume.	Bangkok	CU38-11	VI	*B. coagulans*
Bark of *Lagerstroemia floribunda* Jack ex Blume.	Bangkok	CU68-2	VII	*S. terrae*

Identification methods

Phenotypic and chemotaxonomic characterization

Phenotypic characteristics such as morphological and cultural of the isolates were observed on the cells grew on GYP $CaCO_3$ agar plate after incubated under anaerobic conditions at 37°C for 3 days. Gram reaction, spore formation, gas formation, catalase activity, nitrate reduction, arginine hydrolysis, growth at different temperatures (10- 50°C), at different pH values (4-8.5) and different NaCl concentrations (w/v) were performed as described by Tanasupawat *et al.*(1992; 1998). Acid formation from various carbohydrates were tested as described by Tanasupawat *et al.*(1998). *Meso*-diaminopimelic acid (DAP) in the cell wall peptidoglycan was determined as described by Komagata and Suzuki (1987). The isomer of lactic acid produced by each strain was analyzed by using high-performance liquid chromatography (Chotisubha-anandha, 2008).

Genotypic characterization

The 16S rRNA 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 16S rRNA gene sequence was analyzed by Macrogen®, Korea. Sequence alignment was employed using the BLAST software from the Gen Bank. Multiple alignments of the sequences determined were performed with a program CLUSTAL X (version 1.81). Gaps and ambiguous bases were eliminated prior to construction of a phylogenetic tree. A phylogenetic tree was constructed by the neighbour-joining method (Saitou and Nei, 1987) with the program MEGA version 5.05. The confidence values of individual branches in the phylogenetic tree were determined by using the bootstrap analysis of Felsenstein (1985) based on 1000 replications.

Determination of lactic acid and end product

The isolates grew on GYP slant at 37°C for 48 h were transferred to GYP preculture medium with glucose 10 g/L at 37°C for 48 h. The preculture broth was inoculated into the fermentation medium containing glucose 120 g/L and incubated at 37°C for 72 h. At the end of fermentation, the supernatant was collected for the analysis of lactic acid and the remained glucose using high-performance liquid chromatography (HPLC; Biorad, Aminex HPX-87H ion exclusion organic acid column, 300mm x 7.8mm) maintained at 45°C in a column oven (Shimadzu-CTO-6A). An eluant, 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.

RESULTS AND DISCUSSION

Ten isolates were Gram-positive, catalase (except isolate CU38-1) and oxidase negative lactic acid producing bacteria. They fermented glucose homofermentatively and produced no gas from glucose. They did not grow at 50 °C (except isolate CU38-1) and showed negative reaction to nitrate reduction, hydrolysis of arginine and starch. All isolates were divided into seven groups based on the cell form, spore formation, growth at different temperatures and pH, and NaCl concentrations, peptidoglycan cell wall type and acid production from carbohydrates (Table 2) including 16S rRNA gene sequence analyses.

Group I consisted of four isolates, CU37-12, CU37-21, BK78-1 and NK121. Cells were tetrad forming cocci and non-spore forming. Colonies were smooth, circular, convex, white in colour (0.3-2.9 mm in diameter). They grew at 20-45°C, at pH 4-8.5 and in 7% NaCl. Acid is produced from amygdalin, L-arabinose, D-cellobiose, D-fructose, D-galactose, glucose, lactose, D-maltose, D-mannose, D-ribose and D-xylose. Variable fermentation was found in inulin, D-mannitol, D-melibiose, raffinose, sorbitol and sucrose. *Meso*-diaminopimelic acid was not present in the cell wall peptidoglycan (Table 2). The 16S rRNA gene sequence of all isolates in this group showed 100% similarity to *Pediococcus pentosaceus* DSM 20336^T (Figure 1). Therefore, they were identified as *P. pentosaceus* (Tanasupawat *et al*., 1993).

Group II consisted of an isolate CU72-2. Cells were cocci in chain and non-spore forming. Colonies were smooth, circular, convex, ivory-white in colour (0.8-0.9 mm in diameter). The isolate grew at 25-45°C, at pH 5-8.5 and in 7% NaCl. Acid is produced from amygdalin, L-arabinose, D-fructose, D-galactose, glucose, lactose, D-mannose, D-melibiose, D-ribose, sucrose and D-xylose but did not produce acid from D-cellobiose, inulin, D- maltose, D-mannitol, raffinose, sorbitol and L-sorbose. *meso*- diaminopimelic acid was not present in the cell wall peptidoglycan (Table 2). The 16S rRNA gene sequence of this isolate showed 99.9% similarity to *Enterococcus casseliflavus* CECT 696^T (Figure 1). Therefore, it was identified as *E. casseliflavus* (Naser *et al*., 2006).

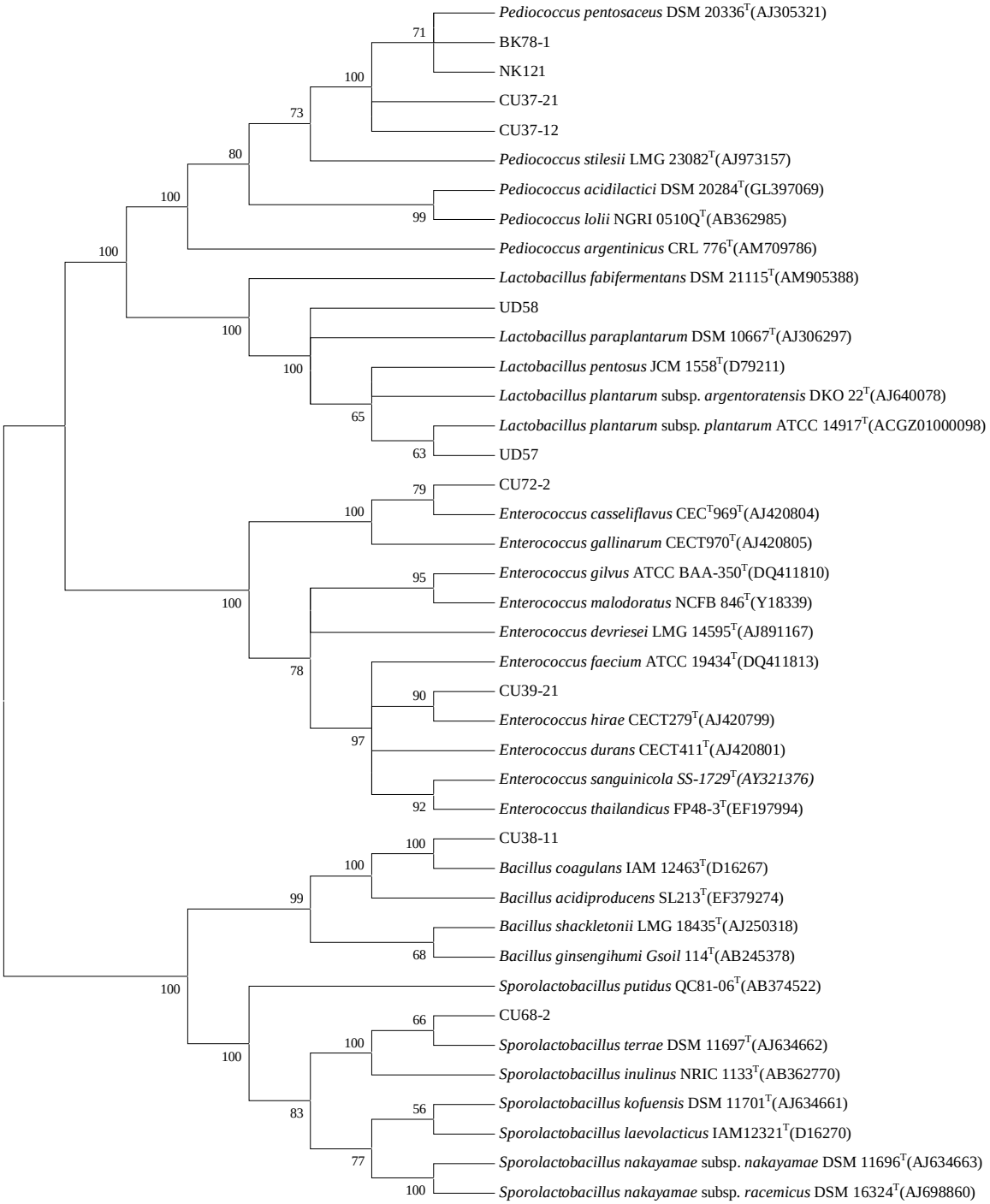

Fig. 1: Phylogenetic tree constructed using the neighbour-joining method showing the position of isolates and related species based on 16S rRNA gene sequences.

Group III consisted of an isolate CU39-21. Cells were cocci in chain and non-spore forming. Colonies were smooth, circular, convex, ivory-white in colour (0.5-1.0 mm in diameter). The isolate grew at 20-45°C, at pH 5-8.5 and in 7% NaCl. Acid is produced from amygdalin, L-arabinose, D-cellobiose, inulin, D-fructose, D-galactose, glucose, lactose, D-mannose, D-melibiose, D-ribose, sucrose and D-xylose but did not produce acid from D-maltose, D-mannitol, raffinose, sorbitol and L-sorbose. *meso*-diaminopimelic acid was not present in the cell wall peptidoglycan (Table 2). The 16S rRNA gene sequence of this isolate showed 100% similarity to *Enterococcus hirae* CECT 279^{T} (Figure 1). Therefore, it was identified as *E. hirae* (Farrow and Collins, 1985). Group IV consisted of one isolate, UD57. Cells were rod shaped and non-spore forming bacteria. Colonies were smooth, circular, convex, ivory-white in colour (1-2.4 mm in diameter). The isolate grew at 25-45°C, at pH 4-8.5 and in 7% NaCl. Acid is produced from amygdalin, L-arabinose, D-cellobiose, D-fructose, D-galactose, glucose, lactose, D-maltose, D-mannitol D-melibiose,

raffinose, D-ribose, sucrose and D-xylose but did not produce acid from inulin, D-mannose, sorbitol and L-sorbose. The cell wall peptidoglycan contained *meso*- diaminopimelic acid (Table 2). The 16S rRNA gene sequence of this isolate showed 100% similarity to *Lactobacillus plantarum* TCC 14917^T (Figure 1). Therefore, it was identified as *L. plantarum* (Curk *et al*., 1996).

Group V consisted of an isolate UD58. Cells were rod shaped and non-spore forming bacteria. Colonies were smooth, circular, ivory-white in colour (0.7-2 mm in diameter). The isolate grew at 20-45 °C, at pH 4-8.5 and in 7% NaCl. Acid is produced from amygdalin, L-arabinose, D-cellobiose, D-fructose, D-galactose, glucose, lactose, D-mannose, D-maltose, D-mannitol D-melibiose, raffinose, D-ribose, L-sorbose, sucrose and D-xylose but did not produce acid from inulin and sorbitol. The cell wall peptidoglycan contained *Meso*-diaminopimelic acid (Table 2). The 16S rRNA gene sequence of the isolate showed 100% similarity to *Lactobacillus paraplantarum* DSM 10667^T (Figure 1). Therefore, it was identified as *L. paraplantarum* (Curk *et al*., 1996).

Group VI consisted of an isolate CU38-11. Cells were rod shaped and spore forming bacteria. Colonies were smooth, circular, convex, ivory-white in colour (0.5-1.3 mm in diameter). This isolate produced catalase, grew at 25-50 °C, at pH 5-8.5 and in 5% NaCl. Acid is produced from amygdalin, L- arabinose, D-cellobiose, D-fructose, D-galactose, glucose, inulin, D-mannose, D-maltose, D-melibiose, raffinose, D-ribose, sucrose and D-xylose but did not produce acid from lactose, D-mannitol, sorbitol and L-sorbose. The cell wall peptidoglycan contained *Meso*-diaminopimelic acid (Table 2). The 16S rRNA gene sequence of this isolate showed 99.5% similarity to *Bacillus coagulans* IAM 12463^T (Figure 1). Therefore, it was identified as *B. coagulans* (Nakamura *et al*., 1988).

Group VII consisted of one isolate, CU68-2. Cells were rod shaped and spore forming bacteria. Colonies were smooth, circular, convex, ivory-white in colour (0.6-1.8 mm in diameter). The isolate grew at 25-45°C, at pH 4-8.5 and in 5% NaCl. Acid is produced from amygdalin, L-arabinose, D-cellobiose, D-fructose, D-galactose, glucose, lactose, D-mannose, D-mannitol, D-maltose, raffinose, D-ribose, sorbitol, L-sorbose, sucrose and D-xylose but did not produce acid from inulin and D-melibiose. The cell wall peptidoglycan contained *meso*-diaminopimelic acid (Table 2). The 16S rRNA gene sequence of this isolate showed 99.8% similarity to *Sporolactobacillus terrae* DSM 11697^T (Figure 1). Therefore, it was identified as *S. terrae* (Yanagida *et al*., 1997).

The results on the qualitative stereoisomer of lactic acid by using HPLC were shown in Table 2 and Table 3. The isolates in Group I, IV and V produced racemic DL-lactic acid but Group VII (CU68-2) could rather produce optically pure D more than L (+)- lactic acid. In contrast, the isolates in Group II, III and VI were optically pure L (+)- lactic acid producers. It was observed that lactic acid of the isolates CU37-12, CU37-21, BK78-1 and NK121 identified as *P. pentosaceus* were 56.62, 56.74, 56.17 and 77.31 g/L, respectively. *E. casseliflavus* CU72-2, *E. hirae* CU39-21, *L. plantarum* UD57, *L. paraplantarum* UD58, and *S. terrae* CU68-2 produced 60.72, 66.04, 70.26, 54.50, and 55.30 g/L of final lactic acid, respectively. In addition, only CU38-11, identified as *B. coagulans* produced the highest 99.75% of optically pure L-lactic acid. L-lactic acid concentration and productivity of this isolate were 89.34 g/L and 1.24 g/L.h from 120 g/L glucose, respectively. In the previous reports, *P. pentosaceus*, *E. casseliflavus*, *E. hirae*, *L. plantarum*, *L. paraplantarum* isolates were isolated from fermented food products (Tanasupawat and Komagata, 2001) while *B. coagulans* was isolated from evaporated milk (Skerman *et al.*, 1980) and *S. terrae* was isolated from soil (Yanagida *et al*., 1997). However, our lactic acid bacterial strains are isolated from soils, plant bark and root that they could produce DL-lactic acid and L-lactic acid which are useful for food preservation, the chemical in pharmaceutical, cosmetic and textile industries.

Table. 2: Phenotypic characteristics of the isolates.

Characteristics	I	II	III	IV	V	VI	VII
Cell form	Cocci	Cocci	Cocci	Rods	Rods	Rods	Rods
Spore formation	-	-	-	-	-	+	+
Growth temp. (°C)	20-45	25-45	20-45	25-45	20-45	25-50	25-45
Growth in NaCl (%)	7	7	7	7	7	5	5
Growth at pH	4-8.5	5-8.5	5-8.5	4-8.5	4-8.5	5-8.5	4-8.5
Acid from:							
D-Cellobiose	+(w1)	-	w	w	w	+	+
Inulin	w(-1)	-	w	-	-	w	-
Lactose	w(+1)	w	w	+	w	-	+
D-Maltose	w(+1)	-	-	+	w	w	+
D-Mannitol	-(+1)	-	-	+	w	-	+
D-Mannose	+	w	+	-	w	+	+
D-Melibiose	w(-1)	w	+	+	w	w	-
Raffinose	-(+2)	-	-	w	+	w	+
D-Ribose	+	+	+	+	+	+	+
Sorbitol	-(w)	-	-	-	-	-	w
L-Sorbose	+(2)	-	-	-	+	-	+
Sucrose	+(-1)	+	+	+	+	+	+
Meso-DAP	-	-	-	+	+	+	+
Isomer of lactic acid	DL	L	L	DL	DL	L	DL

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

Table. 3: Lactic acid and remained glucose of the isolates.

Isolate no.	Group	Lactic acid (g/L)			Remained glucose	
		Final lactic acid (g/L)	% Yield	Productivity(g/L.h)	(g/L)	Isomer (% Optical purity)
CU37-12	I	56.62	61.29	0.79	27.62	DL (ND)
CU37-21	I	56.74	47.28	0.79	0	DL (ND)
BK78-1	I	56.17	70.72	0.78	40.57	DL (ND)
NK121	I	77.31	75.11	1.07	17.07	DL (ND)
CU72-2	II	60.72	73.64	0.84	37.55	L (96.78)
CU39-21	III	66.04	70.00	0.92	25.66	L (66.82)
UD57	IV	70.26	72.08	0.98	22.52	DL (ND)
UD58	V	54.50	45.88	0.76	1.20	DL (ND)
CU38-11	VI	89.34	74.45	1.24	0	L (99.75)
CU68-2	VII	55.30	46.08	0.77	0	DL (ND)

ND, not determined.

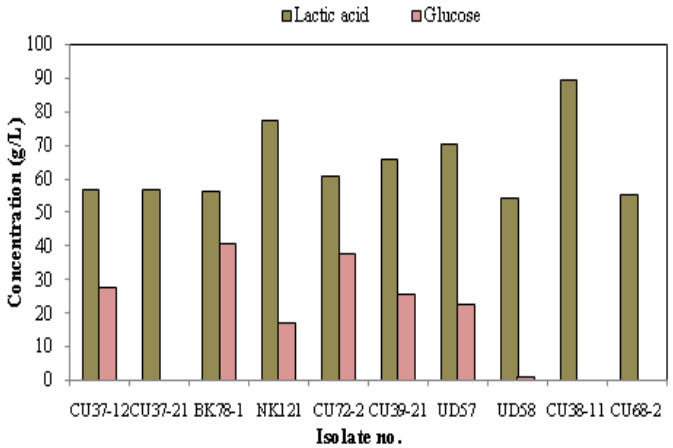

Fig. 2: Lactic acid fermentation and remained glucose of isolates.

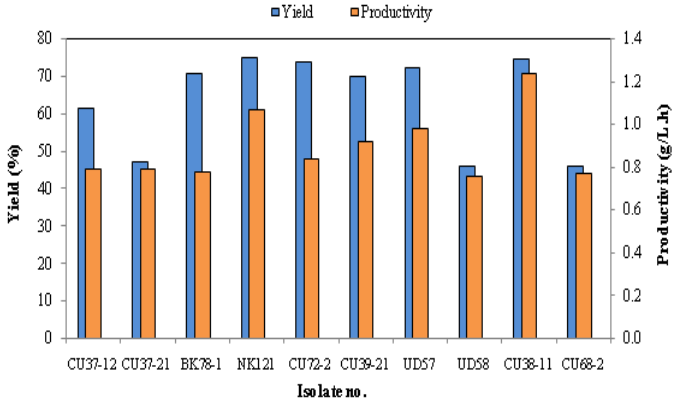

Fig. 3: Yields of lactic acid and productivities of isolates. Each experiment was performed at 37 °C, 72 h in GYP broth containing 120 g/L glucose.

CONCLUSION

In this present study, we found that lactic acid producing bacteria distributed in diverse samples. *P. pentosaceus* isolates are isolated from soils, plant bark and root while *E. casseliflavus*, *E. hirae*, *Lactobacillus. plantarum*, *L. paraplantarum*, *B. coagulans* and *S. terrae* isolates are distributed in plant bark. The isolates in Group I, IV, V and VII produced DL-lactic acid and the isolates in in Group II, III and VI produced L (+)- lactic acid. However, *B. coagulans* isolate in Group VI could produce high optically pure L-lactic acid with 99.75 % that will be interesting for the further study.

ACKNOWLEDGEMENTS

The authors thank ‘‘The 90th Anniversary of Chulalongkorn University Fund, 2011 (Ratchadaphiseksomphot Endowment Fund)’’ for financial support.

REFERENCES

Axelsson L. Lactic acid bacteria: classification and physiology. In: Salminen S, von Wright, A, Ouwehand A (Eds.) Lactic Acid Bacteria, Microbiology and Functional Aspects. Marcel Dekker, Inc., New York. 2004; pp. 1-66.

Chotisubha-anandha N. Production of L (+) lactic acid by immobilized *Rhizophus oryzae* in a static bed bioreactor. A thesis submitted in partial fulfillment of the requirement for degree of master science program in biotechnology, Faculty of Science, Chulalongkorn University. 2008; 49.

Curk MC, Hubert JC, Bringe lF. *Lactobacillus paraplantarum* sp. nov., a new species related to *Lactobacillus plantarum*. Int J Syst Bacteriol. 1996; 46: 595-598.

Farrow JAE, Collins MD. *Enterococcus hirae*, a new species that includes amino acid assay strain NCDO 1258 and strains causing growth depression in young chickens. Int J Syst Bacteriol. 1985; 35: 73-75.

Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution.1985; 39: 783–791.

John RP, Anisha GS, Nampoothiri KM, Pandey A. Direct lactic acid fermentation: focus on simultaneous saccharification and lactic acid production. Biotechnol Advan.2009; 27: 145-172.

Kandler O, Weiss N. Genus *Lactobacillus* Beijerinck 1901, 212 AL. In: Sneath, HA, Mair NS, Sharpe ME, Holt JG. (Eds.) Bergey’s Manual of Systematic Bacteriology, Vol. 2. Baltimore: Williams & Wilkins. 1986; pp. 1209-1234.

Komagata K, Suzuki K. Lipid and cell-wall analysis in bacterial systematic. In: Colwell, RR, Grigorava R. (Ed.) Methods in Microbiology. London: Academic Press Limited. 1987 pp. 161-207.

Nakamura LK, Blumenstock I, Clau SD. Taxonomic Study of *Bacillus coagulans Hammer* 1915 with a Proposal for *Bacillus srnithii* sp. nov. Int J Syst Bacteriol. 1988; 38: 63-73.

Naser SM, Vancanneyt M, Hoste B, Snauwaert C, Vandemeulebroecke K, Swings J. Reclassification of *Enterococcus flavescens* Pompei et al. 1992 as a later synonym of *Enterococcus casseliflavus*(ex Vaughan et al.1979) Collins et al. 1984 and *Enterococcus saccharominimus* Vancanneyt et al. 2004 as a later synonym of Enterococcus *italicus* Fortina et al.2004. Int J Syst Evol Microbiol. 2006; 56: 413–416.

Saitou N, Nei M. The neighboring-joining method: a new method for reconstructing phylogenetic trees**.** Mol Biol Evol. 1987; 4:406-425.

Skerman, VBD, McGowan, V, Sneath, P H A. Approved lists of bacterial names. Int J Syst. Bacteriol. 1980; 30: 225-420.

Tanasupawat S, Komagata K. Lactic acid bacteria in fermented foods in Southeast Asia. In: Nga BH, Tan HM, Suzuki K. (Eds.) Microbial Diversity in Asia: Technology and Prospects, World Scientific Publishing Co. Pte. Ltd. Singapore. 2001; pp. 43-59.

Tanasupawat S, Okada S, Kozaq M, Komagata K. Characterization of *Pediococcus pentosaceus* and *Pediococcus acidilactici* strains and replacement of the type strain of *P. acidilactici* with the proposed neotype DSM 20284: request opinion. Int J Syst Bacteriol. 1993; 43: 860-863.

Tanasupawat S, Okada S, Komagata K. Lactic acid bacteria found in fermented fish in Thailand. J Gen Appl Microbiol. 1998; 44: 193-200.

Tanasupawat S, Thongsanit J, Okada S, Komagata K. Lactic acid bacteria isolated from soy sauce mash in Thailand. J Gen Appl Microbiol. 2002; 48: 201-209.

Tanasupawat S, Thongsanit J, Okada S, Komagata K. Lactic acid bacteria isolated from soy sauce mash in Thailand. J Gen Appl Microbiol. 2002; 48: 201-209.

Wee YJ, Kim JN, Ryu HW. Biotechnological production of lactic acid and its recent applications. Food Technol Biotechnol. 2006; 44: 163-172.

Xu TT, Bai ZZ, Wang LJ, He BF. Breeding of D(–)-lactic acid high producing strain by low-energy ion implantation and preliminary analysis of related metabolism. Appl Biochem Biotechnol. 2008; 160: 314-321.

Yanagida F, Suzuki KI, Kozaki M, Komagata K. Proposal of *Sporolacto bacillus nakayamae* sub sp. *nakayamae* sp. nov., *Sporolacto bacillus nakayamae* sub sp. *racemicus* subsp. nov., *Sporolactobacillus terrae* sp. nov., *Sporolactobacillus kofuensis* sp. nov. and *Sporolacto bacillus lactosus* sp. nov. Int J Syst Bacteriol.1997; 47: 499-504.

How to cite this article:

Budsabathip Prasirtsak, Somboon Tanasupawat, Ruethairat Boonsombat, Kentaro Kodama and Nuttha Thongchul., Characterization of lactic acid producing bacteria from Thai sources. J App Pharm Sci. 2013; 3 (01): 033-038.