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Identification of lactic acid bacteria from fermented tea leaves (*miang*) in Thailand and proposals of *Lactobacillus thailandensis* sp. nov., *Lactobacillus camelliae* sp. nov., and *Pediococcus siamensis* sp. nov.

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(Received June 26, 2006; Accepted November 27, 2006)

Eighteen rod-shaped homofermentatives, six heterofermentatives, and a coccal homofermentative lactic acid bacteria were isolated from fermented tea leaves (*miang*) produced in the northern part of Thailand. The isolates were placed in a monophyletic cluster consisting of *Lactobacillus* and *Pediococcus* species. They were divided into seven groups by phenotypic and chemotaxonomic characteristics, DNA-DNA similarity, and 16S rRNA gene sequences. Groups I to VI belonged to *Lactobacillus* and Group VII to *Pediococcus*. All of the strains tested produced D-lactic acid but those in Group IV produced L-lactic acid. The strains tested in Groups I, II and V had meso-diaminopimelic acid in the cell wall. Six strains in Group I were identified as *Lactobacillus pantheris*; five strains in Group II as *Lactobacillus pentosus*; and four strains in Group V as *Lactobacillus suebicus*. Two strains in Group VI showed high DNA-DNA similarity for each other and MCH4-2 was closest to *Lactobacillus fermentum* CECT 562^T with 99.5% of 16S rRNA gene sequence similarity. Five strains in Group III are proposed as *Lactobacillus thailandensis* sp. nov., and MCH5-2^T (BCC 21235^T=JCM 13996^T=NRIC 0671^T=PCU 272^T) is the type strain which has 49 mol% G+C of DNA. Two strains in Group IV are proposed as *Lactobacillus camelliae* sp. nov., and the type strain is MCH3-1^T (BCC 21233^T=JCM 13995^T=NRIC 0672^T=PCU 273^T) which has 51.9 mol% G+C of DNA. One strain in Group VII is proposed as *Pediococcus siamensis* sp. nov., and MCH3-2^T (BCC 21234^T=JCM 13997^T=NRIC 0675^T=PCU 274^T) is the type strain which has 42 mol% G+C of DNA.

Key Words—fermented tea leaves; lactic acid bacteria; *Lactobacillus camelliae*; *Lactobacillus thailandensis*; *Pediococcus siamensis*

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Introduction

Miang (fermented tea leaves) is one of the fermented products in the northern part of Thailand. Young tea leaves are collected, steamed, wrapped tightly in individual bundles, packed into containers,

pressed tightly and weighted down, covered with banana leaves and plastic sheets, and fermented for 4–7 days, or 1 year for mature tea leaves (Appleton et al., 1978–1980). It is consumed as a snack. Lactic acid bacteria in *miang* were already reported, and they were *Lactobacillus plantarum*, *L. pentosus*, *L. vacci-nostercus*, *Lactobacillus* sp., *Enterococcus cas-seliflavus*, and *Enterococcus* sp. (Okada et al., 1979, 1986; Tanasupawat et al., 1992a, b; Tanasupawat and Komagata, 1995). However, there are still a number of unidentified lactic acid bacteria in the product.

This study deals with the identification of newly isolated lactic acid bacteria from *miang*, and proposals of *Lactobacillus thailandensis* sp. nov., *Lactobacillus camelliae* sp. nov., and *Pediococcus siamensis* sp. nov. sp.

Materials and Methods

Isolation methods. Lactic acid bacteria were isolated by an enrichment culture approach from six samples of fermented tea leaves (*miang*) collected at the markets in Chiang Mai and Bangkok provinces. Pure cultures were obtained by streaking cultured cells on MRS-CaCO₃ agar plates (De Man et al., 1960). Twenty-five isolates and each of the type strains of related *Lactobacillus* species are used (Tables 1 and 4).

Identification methods. Phenotypic characterization: The morphological, cultural, physiological, and biochemical characteristics and the isomers of lactic acid produced were determined as reported previously (Okada et al., 1978; Tanasupawat et al., 1998).

Cell wall peptidoglycan type: The *meso*-diaminopimelic acid (*meso*-DAP) and lysine of cell wall were determined as described previously (Komagata and Suzuki, 1987; Tanasupawat et al., 1993).

DNA base composition: DNAs were isolated and purified as reported previously (Saito and Miura, 1963). The medium supplemented with 0.8–1.5% glycine was used for strains with difficulty in isolation of DNA (Yamada and Komagata, 1970). The purified DNA was hydrolyzed to nucleosides as described previously and the hydrolysate was applied to reversed phase high-performance liquid chromatography (HPLC) (Tamaoka and Komagata, 1984).

DNA-DNA hybridization: DNAs were isolated and purified by the method reported previously (Saito and Miura, 1963; Yamada and Komagata, 1970). Photobiotin labeling DNA-DNA hybridization was carried out in

2×SSC (Saline trisodium citrate) and 50% formamide solution at 40 or 45°C for 15 h (Ezaki et al., 1989). DNA-DNA similarity was determined by using the colorimetric method as reported (Tanasupawat et al., 2000).

16S rRNA sequence and phylogenetic analysis: The 16S rRNA gene sequence was determined as described previously (Tanasupawat et al., 2004). The sequences determined (1,417–1,535 bases) were aligned with the selected sequences obtained from the GenBank/EMBL/DDBJ database employing CLUSTAL_X (Thompson et al., 1997). The alignment was edited to remove gaps and ambiguous nucleotides prior to construction of a phylogenetic tree. The phylogenetic tree was constructed by using the neighbor-joining method (Saitou and Nei, 1987) in the MEGA program version 2.1 (Kumar et al., 2001). The confidence values of branches of the phylogenetic tree were determined by using bootstrap analyses (Felsenstein, 1985) based on 1,000 resamplings. Sequences of 16S rRNA were submitted to DDBJ and GenBank with the following accession numbers: MCH4-2 (AB257865), MCH2-2 (AB257866), MCH5-2^T (AB257863), FP37-1 (AB257867), MCH3-1^T (AB257864), and MCH3-2^T (AB258357).

Results and Discussion

Of a total of twenty-five strains isolated from 6 samples of *miang* 24 were rod-shaped and 1 sphere-shaped (Table 1). The rod-shaped and sphere-shaped strains were widely distributed in *miang*. All the strains were placed in a monophyletic cluster consisting of *Lactobacillus* and *Pediococcus* species. They were Gram-positive, catalase negative, and they appeared singly, in pairs, in chains, or in tetrads. Colonies on MRS agar plate were circular, low convex with an entire margin. The strains were divided into 7 groups by phenotypic characteristics, isomers of lactic acid, cell wall composition, DNA base composition and DNA-DNA similarity (Tables 2, 3, and 4). All the representative strains produced DL-lactic acid, but the strain in Group IV produced L-lactic acid. Strains of Groups I, II and V had *meso*-diaminopimelic acid (DAP) in the cell wall (Table 2).

The rod-shaped strains, FP37-1 (Group I) and MCH5-2^T (Group III) showed, 99.1 and 98.7% of 16S rDNA sequence similarity to *L. pantheris* LMG 21017^T while MCH4-2 (Group VI), MCH3-1^T (Group IV), and

Table 1. Sources of collected samples, strain no., and identification.

Sample no./Province	Strain no.	Group	Identification
1. Chiang Mai	FP37-1 (=NRIC 0415)	I	<i>Lactobacillus pantheris</i>
2. Bangkok (Chatuchak market)	MCH1-1 (=NRIC 0666), MCH1-2, MCH1-3, MCH1-4 (=NRIC 0667), MCH1-6	I	<i>Lactobacillus pantheris</i>
	MCH1-7 (=NRIC 0668)	II	<i>Lactobacillus pentosus</i>
3. Chiang Mai (Tapair market)	MCH2-5 (=NRIC 0669)	II	<i>Lactobacillus pentosus</i>
	MCH2-1 (=NRIC 0673), MCH2-2, MCH2-3, MCH2-4	V	<i>Lactobacillus suebicus</i>
4. Chiang Mai (Tapair market)	MCH3-1 ^T (=NRIC 0672 ^T), MCH3-3	IV	<i>Lactobacillus camelliae</i> sp. nov.
	MCH3-2 ^T (=NRIC 0675 ^T)	VII	<i>Pediococcus siamensis</i> sp. nov.
5. Chiang Mai (Varoroch market)	MCH4-3	II	<i>Lactobacillus pentosus</i>
	MCH4-1 (=NRIC 0670), MCH4-4	III	<i>Lactobacillus thailandensis</i> sp. nov.
	MCH4-2 (=NRIC 0674), MCH4-5	VI	<i>Lactobacillus fermentum</i>
6. Chiang Mai (Varoroch market)	MCH5-5, MCH5-6	II	<i>Lactobacillus pentosus</i>
	MCH5-2 ^T (=NRIC 0671 ^T), MCH5-4, MCH5-9	III	<i>Lactobacillus thailandensis</i> sp. nov.

MCH2-2 (Group V) showed 99.5, 96.1 and 98.6% of 16S rRNA gene sequence similarity to *L. fermentum* CECT 562^T, *L. manihotivorus* LMG 18010^T and *L. suebicus* CECT 5917^T, respectively. They were placed in a monophyletic cluster consisting of *Lactobacillus* species (Fig. 1). The coccal strain, MCH3-2^T (Group VII) showed 96.8% of 16S rRNA gene sequence similarity to *Pediococcus cellicola* Z-8^T that was placed in a monophyletic cluster consisting of *Pediococcus* species as shown in Fig. 1.

Group I contained six strains (FP37-1, MCH1-1, MCH1-2, MCH1-3, MCH1-4, MCH1-6). These strains had DAP in the cell wall. All the strains produced acid from cellobiose, esculin, D-fructose, D-galactose, D-glucose, lactose, D-mannose and salicin (Tables 2 and 3). A representative strain, FP37-1 showed the high degree of DNA-DNA similarity (over 77.9%) to strains in the group, and the strains showed the high degree of DNA-DNA similarity (over 81.6%) to *Lactobacillus pan-*

theris NRIC 0613^T (=LMG 21017^T). The strains in this group showed a low degree of DNA-DNA similarity (less than 11.2%) to *L. plantarum* NRIC 1067^T, *L. pentosus* NRIC 1069^T and *L. paraplantarum* CNRZ 1885^T (Table 4). Furthermore, strain FP37-1 showed 99.1% similarity of 16S rRNA sequence to *Lactobacillus pantheris* LMG 21017^T, and it was located in a monophyletic cluster (Fig. 1). The DNA G+C content of strains tested ranged from 50 to 52 mol%, which was close to that of *Lactobacillus pantheris* NRIC 0613^T (Table 4). Therefore, the isolates in this group were identified as *L. pantheris* (Liu and Dong, 2002; Wayne et al., 1987). *L. pantheris* was originally established based on two strains, and was reported to produce D-lactic acid (Liu and Dong, 2002). In this study, *L. pantheris* NRIC 0613^T and our isolates produced DL-lactic acid and had meso-diaminopimelic acid (DAP) in the cell wall. In addition, strain FP37-1 required riboflavin, biotin, niacin, calcium-pantothenate, and folic acid for

Table 2. General characteristics of strains.

Characteristics	Group I (6) ^a	Group II (5)	Group III (5)	Group IV (2)	Group V (4)	Group VI (2)	Group VII (1)
Cell form	Rods	Rods	Rods	Rods	Rods	Rods	Cocci
Cell size (μm)	0.4–1×2–6	0.8–1×2–6	0.5–0.6×2–6	0.6–1×2–5	0.5–0.8×2–5	0.6–1×3–8	0.5–0.7
Gas from glucose	–	–	–	–	+	+	–
Gas from gluconate	–	+	–	–	+	+	–
Arginine hydrolysis	–	–	–	–	–	+	–
Esculin hydrolysis	+	+	+	+	–	–	+
Nitrate reduction	–	–	–	–	–	+	–
Reaction in litmus milk:							
Acidification	+	+(–2)	–	–	–	–	–
Coagulation	–(+3)	+(–2)	–	–	–	–	–
Reduction	–(+2)	+(–2)	–	–	–	–	–
Growth at 15°C	+	+	w	+	w	–	+
45°C	–	–	–	–	–	+	+
Growth at pH 3.5	–	+	–	–	–	+	–
pH 8.0	+(–1)	+	+	+	+	+	+
pH 8.5	–(+1)	+	+	–	–	–	+
pH 9.0	–	–(+1)	–	–	–	–	–
Growth in 4% NaCl	+(–1)	+	+	–	–	+	+
6% NaCl	+(–1)	+	–(w1)	–	–	–	–
8% NaCl	–	+(–1)	–	–	–	–	–
<i>meso</i> -DAP in cell							
wall peptidoglycan	+	+	–	–	+	–	–
Isomer of lactic acid	DL	DL	DL	L	DL	DL	DL

+, positive; w, weak positive; –, negative reaction.

^a Number of strains.

Numbers in parentheses indicate the number of strains showing the reaction.

growth but not thiamine, *p*-aminobenzoic acid, or pyridoxal. The strain FP37-1 had the straight chain fatty acids of C_{18:1} and C_{16:0} as major components but had no cyclopropane acids as reported by Tanasupawat et al. (1992a). The detailed description of this species mentioned here will be useful for their circumscription.

Group II included five strains (MCH1-7, MCH2-5, MCH4-3, MCH5-5, and MCH5-6). They had *meso*-diaminopimelic acid in the cell wall, and the tested strains produced DL-lactic acid. All the strains produced acid from D-amydalin, cellobiose, esculin, D-fructose, D-glucose, maltose, D-mannose, salicin, sucrose, and trehalose (Tables 2 and 3). All the strains showed a high degree of DNA-DNA similarity (over 95.2%) to *L. pentosus* NRIC 1069^T, and they were identified as *L. pentosus* (Hammes et al., 1992; Tanasupawat et al., 1992a; Wayne et al., 1987; Zanoni et al., 1987).

Group III contained five strains (MCH4-1, MCH4-4, MCH5-2^T, MCH5-4, and MCH5-9). These strains did not have *meso*-diaminopimelic acid in the cell wall. All the strains produced acid from esculin, D-fructose, D-galactose, D-glucose, D-mannose and salicin (Tables 2 and 3). Weak growth was observed at 15 but not at 45°C. They were separated from the strains in Groups I, II, IV, V and VI by phenotypic characteristics (Tables 2 and 3). The representative strain in this group, MCH5-2^T showed 98.7% similarity of 16S rRNA gene sequence to *L. pantheris* LMG 21017^T (Liu and Dong, 2002) (Fig. 1). Strain MCH5-2^T contained L-lysine-aspartate in the cell wall. However, they showed a low degree of DNA-DNA similarity (less than 12.5%) to *L. pantheris* NRIC 0613^T (Table 4). They were differentiated from *L. pantheris* NRIC 0613^T by the acid production from D-amydalin, maltose, and trehalose, and *meso*-diaminopimelic acid in the cell wall (Table 5).

Table 3. Acid from carbohydrates of strains.

Carbohydrates	Group I (6) ^a	Group II (5)	Group III (5)	Group IV (2)	Group V (4)	Group VI (2)	Group VII (1)
D-Amygdalin	+(-1)	+	+(-1)	+	-	-	+
L-Arabinose	-	+(-2)	-(+1)	+	+	+	-
Cellobiose	+	+	+	+	-	-	+
Esculin	+	+	+	+	-	-	+
D-Fructose	+	+	+	+	-	+	+
D-Galactose	+	+(-2)	+	+	-	+	+
Gluconate	-	+(w2)	-	-	+(w1)	w	-
Lactose	+	+(-2)	+(-1)	-	-	+	+
Maltose	+(-2)	+	-	+	+	+	-
D-Mannitol	-	+(w2)	-	+	-	-	-
D-Mannose	+	+	+	+	-	+	+
Melibiose	-	+(-2)	-	-	+(w3)	w	-
α-Methyl-D-glucoside	-	+(-2)	-	-	-	-	-
Raffinose	-	+(-2)	-	-	-	+	-
Rhamnose	w(-2)	-(w1)	-	-	-	-	-
Ribose	-	+(-1)	-	-	-	w	-
Salicin	+	+	+	+	-	-	+
Sorbitol	-	-(+2)	-	-	-	-	-
Sucrose	-	+	-	-	-	+	-
Trehalose	+(-1)	+	-	-	-	+	+
D-Xylose	-	+(w2)	-	+	+	+	-

+, positive; w, weak positive; -, negative reaction.

^a Number of strains.

Numbers in parentheses indicate the number of strains showing the reaction. All are positive for glucose but negative for glycerol, inulin, and melezitose.

Therefore, a new species, *Lactobacillus thailandensis* sp. nov., is proposed for this group.

Group IV contained two strains (MCH3-1^T and MCH3-3). All the strains produced acid from D-amydalin, L-arabinose, cellobiose, esculin, D-fructose, D-galactose, D-glucose, maltose, D-mannitol, D-mannose, salicin, and D-xylose (Tables 2 and 3). Growth was observed at 15 but not at 45°C. The representative strain, MCH3-1^T (Group IV), was closely related to *L. manihotivorans* LMG 18010^T, *L. zae* ATCC 15820^T, and *L. casei* ATCC 334^T with 96.1, 95.4, and 95.3% of 16S rRNA gene sequence similarity, respectively (Fig. 1). The two strains showed a low degree of DNA-DNA similarity (less than 28.5%) to *Lactobacillus manihotivorans* JCM 12514^T (=LMG 18010^T) (Table 4). The strains were separated from the strains in Groups I, II, III, V and VI by phenotypic characteristics (Tables 2 and 3). Strain MCH3-1^T contained L-lysine-aspartate in the cell wall. Further, they were differentiated from *L.*

manihotivorans LMG 18010^T, *L. casei* ATCC 334^T, and *L. zae* ATCC 15820^T (Dicks et al., 1996; Kandler and Weiss, 1986; Morlon-Guyot et al., 1998) by the growth at 45°C; acid production from L-arabinose, lactose, mannitol, melibiose, raffinose, sucrose, trehalose and D-xylose; and DNA G+C content (Table 5). Therefore a new species, *Lactobacillus camelliae* sp. nov., is proposed for this group.

Group V contained four strains (MCH2-1, MCH2-2, MCH2-3, and MCH2-4). All the strains produced gas from glucose heterofermentatively and produced acid from L-arabinose, D-glucose, maltose, and D-xylose. The strains tested had *meso*-diaminopimelic acid in the cell wall. The strains produced DL-lactic acid and had DNA G+C content from 39 to 39.3 mol% (Tables 2 and 3). The representative strain in this group, MCH2-2, was related to *L. suebicus* CECT 5917^T with 98.6% similarity of 16S rRNA gene sequence (Fig. 1). However, MCH2-2 and other strains showed a high degree

Table 4. DNA base composition and DNA-DNA similarity of strains.

Species	Strains	G+C content (mol%)	% DNA similarity with labeled strains	
Group I			FP37-1	NRIC 0613 ^T
	FP 37-1	52	100.0	92.8
	MCH1-1	51	92.6	88.8
	MCH1-2		91.4	89.2
	MCH1-3	50	93.2	81.6
	MCH1-4		90.9	88.6
	MCH1-6		77.9	73.1
<i>L. plantarum</i>	NRIC 1067 ^T		5.4	6.6
<i>L. pentosus</i>	NRIC 1069 ^T		5	6.5
<i>L. paraplantarum</i>	CNRZ 1885 ^T		11.2	6.7
<i>L. pantheris</i>	NRIC 0613 ^T		99.2	100
Group II			NRIC 1069 ^T	MCH5-6
	MCH1-7		100.3	93.1
	MCH2-5		101.3	89.5
	MCH4-3		95.2	97.7
	MCH5-5		102.8	102.9
	MCH5-6		100.3	100
<i>L. pentosus</i>	NRIC 1069 ^T		100	76.2
<i>L. plantarum</i>	NRIC 1067 ^T		31.3	32.4
<i>L. paraplantarum</i>	CNRZ 1885 ^T		35.9	49.2
Group III			MCH5-2 ^T	NRIC 0613 ^T
	MCH4-1	49.4	89.7	4.7
	MCH4-4		92.4	11.8
	MCH5-2 ^T	49.0	100.0	7.0
	MCH5-4	50.3	98.3	12.5
	MCH5-9	49.7	95.0	9.3
<i>L. pantheris</i>	NRIC 0613 ^T		10.8	100
Group IV			MCH3-1 ^T	JCM 12514 ^T
	MCH3-1 ^T	51.9	100.0	22.9
	MCH3-3	51.5	101.2	28.5
<i>L. manihotivorans</i>	JCM 12514 ^T		12.8	100
Group V			MCH2-2	NRIC 1637 ^T
	MCH2-1		93.4	78.8
	MCH2-2	39.3	100.0	82.7
	MCH2-3		109.6	102.2
	MCH2-4	39.0	86.0	85.2
<i>L. suebicus</i>	NRIC 1637 ^T		94.7	100
Group VI			MCH4-2	
	MCH4-2	50.2	100.0	
	MCH4-5		96.7	

Table 5. Differential characteristics of *L. thailandensis*, *L. camelliae* and closely related species.

Characteristics	1	2	3 ^a	4 ^b	5 ^c	6 ^d
D-Amygdalin	+	+	–	+	–	+
L-Arabinose	–	+	–	–	+	–
Gluconate	–	–	–	–	+	+
Lactose	+	–	+	+	+	+
Maltose	–	+	+	+	+	+
Mannitol	–	+	–	–	+	+
Melezitose	–	–	–	–	+	+
Melibiose	–	–	–	+	–	–
Raffinose	–	–	–	+	–	–
Rhamnose	–	–	–	–	–	+
Ribose	–	–	–	–	+	+
Sorbitol	–	–	–	–	+	–
Sucrose	–	–	–	+	+	+
Trehalose	–	–	+	+	+	+
D-Xylose	–	+	–	–	–	–
NH ₃ from arginine	–	–	–	ND	–	–
Lactic acid isomer	DL	L	DL	L	L	–
Growth at 45°C	–	–	–	+	ND	+
meso-DAP in the cell wall	–	–	+	–	–	–
DNA G+C (mol%)	49	51.9	52.7	48.4	45–47	48–49

Abbreviations: 1, *L. thailandensis* MCH5-2^T; 2, *L. camelliae* MCH3-1^T; 3, *L. pantheris* NRIC 0163^T; 4, *L. manihotivorans* JCM 12514^T; 5, *L. casei* ATCC 334^T; 6, *L. zeae* ATCC 15820^T.

+, positive; –, negative reaction; ND, no data.

^aData from Liu and Dong (2002); ^bdata from Morlon-Guyot et al. (1998); ^cdata from Kandler and Weiss (1986); ^ddata from Dicks et al. (1996).

of DNA-DNA similarity (over 78.8%) to that of *Lactobacillus suebicus* NRIC 1637^T (=DSM 5007^T) (Table 4). Thus, they were identified as *Lactobacillus suebicus* (Kleynmans et al., 1989).

Group VI contained two strains (MCH4-2 and MCH4-5). The strains produced gas from D-glucose heterofermentatively (Table 1). They hydrolyzed arginine, reduced nitrate, grew at 45°C and in 4% NaCl, and produced acid from L-arabinose, D-fructose, D-galactose, D-glucose, lactose, maltose, D-mannose, raffinose, sucrose, trehalose and D-xylose (Tables 2 and 3). Their phenotypic characteristics and DNA G+C content (50.2 mol%) were the same as those described by Tanasupawat et al. (1993). In addition, a representative strain, MCH4-2, was closely related to *L. fermentum* CECT 562^T with 99.5% similarity of 16S rRNA gene sequence (Fig. 1). MCH4-2 and MCH4-5

Fig. 1. Phylogenetic tree, based on 16S rRNA gene sequences showing the relationships among strains FP37-1, MCH2-2, MCH3-1^T, MCH3-2^T, MCH4-2, MCH5-2^T, *Lactobacillus* species and *Pediococcus* species. The branching pattern was generated by the neighbor-joining method. Bootstrap percentages above 51%, based on 1,000 replications, are shown at the nodes. Bar, 1 substitution per 100 nucleotide positions.

showed a high DNA-DNA similarity (96.7%) to each other (Table 4). Therefore, they were identified as *L. fermentum* (Tanasupawat et al., 1993).

Group VII contained one strain, MCH3-2^T. It did not have *meso*-diaminopimelic acid but contained L-lysine-aspartate in the cell wall. Growth was observed at 15 and 45°C. Strain MCH3-2^T was closely related to *Pediococcus cellicola* Z-8^T, *P. damnosus* DSM 20331^T and *P. inopinatus* LMG 11409^T with 96.8, 96.4, and 96.2% of 16S rRNA gene sequence similarity, respectively (Fig. 1). This strain was differentiated from *P. cellicola* Z-8^T (Zhang et al., 2005) by acid production from L-arabinose, maltose, rhamnose, D-ribose, sucrose, and D-xylose; DNA G+C content (42 mol%), and 16S rRNA gene sequence similarity (Table 6, Fig. 1). Therefore a new species, *Pediococcus siamensis* sp. nov., is proposed for this group.

Description of *Lactobacillus thailandensis* sp. nov.
Lactobacillus thailandensis (thai. lan' den. sis. M. L. fem. adj. *thailandensis*, pertaining to Thailand, where

Table 6. Differential characteristics of *P. siamensis* and related *Pediococcus* species.

Characteristics	1	2 ^a	3 ^b	4 ^b
L-Arabinose	—	+	—	—
Lactose	+	+	—	+
Maltose	—	+	D+	+
Melezitose	—	—	D+	—
Rhamnose	—	+	—	—
Ribose	—	+	—	—
Sucrose	—	+	D+	D+
D-Xylose	—	+	—	—
α-Methyl-D-glucoside	—	—	D+	D+
Growth at pH 7.0	+	+	—	+
Growth at 30°C	+	+	—	+
DNA G+C (mol%)	42	37	40.0 ^c	ND

Abbreviations: 1, *P. siamensis* MCH3-2^T; 2, *P. cellicola* Z-8^T; 3, *P. damnosus*; 4, *P. inopinatus*.

+, positive; D+, some strains positive; —, negative; ND, no data.

^aData from Zhang et al. (2005); ^bdata from Back (1978); ^cdata from Tanasupawat and Komagata (1988).

the strains were isolated).

Cells are Gram-positive, homofermentative rods, measuring 0.5 to 0.6 by 2 to 6 μm , occurring singly, in pairs or in chains. Colonies are circular, white, low convex with entire margin (0.3 to 1.1 mm in diameter) and nonpigmented. Their phenotypic and chemotaxonomic characteristics including DNA-DNA similarity, separating this species from other species are shown in Tables 2, 3, 4 and 5. The type strain is MCH5-2^T (BCC 21235^T=JCM 13996^T=NRIC 0671^T=PCU 272^T), which has 49 mol% of DNA G+C content.

Description of *Lactobacillus camelliae* sp. nov.

Lactobacillus camelliae (ca. mel. li'. ae N. L. gen. n. *camelliae* of *Camellia*, fermented tea (*Camellia sinensis*) leaves, a source of the strains isolated).

Cells are Gram-positive, homofermentative rods, measuring 0.6 to 1 by 2 to 5 μm , occurring singly, in pairs or in chains. Colonies are circular, white, low convex with entire margin (0.3 to 0.8 mm in diameter) and nonpigmented. Their phenotypic and chemotaxonomic characteristics including DNA-DNA similarity, separating this species from other species are shown in Tables 2, 3, 4 and 5. The type strain is MCH3-1^T (BCC 21233^T=JCM 13995^T=NRIC 0672^T=PCU 273^T), which has 51.9 mol% of DNA G+C content.

Description of *Pediococcus siamensis* sp. nov.

Pediococcus siamensis (si. am. en' sis. M. L. fem. adj. *siamensis*, pertaining to Thailand, where the strain was isolated).

Cells are Gram-positive, homofermentative cocci, measuring 0.5 to 0.7 μm in diameter, occurring singly, in pairs or in tetrads. Colonies are circular, white, low convex with entire margin (less than 0.5 mm in diameter) and nonpigmented. Their phenotypic and chemotaxonomic characteristics separating this species from other species are shown in Tables 2, 3, and 6. The type strain is MCH3-2^T (BCC 21234^T=JCM 13997^T=NRIC 0675^T=PCU 274^T), which has 42 mol% of DNA G+C content.

In the previous papers, we reported the isolation of strains of *Lactobacillus plantarum*, *L. pentosus*, *L. vaccinostercus*, *Lactobacillus* sp., *Enterococcus casseliflavus*, and *Enterococcus* sp. from *miang* (Okada et al., 1979, 1986; Tanasupawat et al., 1992a, b; Tanasupawat and Komagata, 1995). Further, we found the strains of *L. pantheris*, *L. pentosus*, *L. suebicus*, and *L.*

fermentum, and the three novel species, *L. thailandensis*, *L. camelliae*, and *P. siamensis* in *miang*. Consequently, lactic acid bacteria would play roles in the acid production and preservation of *miang*. In addition, the new *Lactobacillus* species are close to *L. pantheris* from faeces of jaguar and *L. manihotivorans* strains from sour fermented cassava starch (Liu and Dong, 2002; Morlon-Guyot et al., 1998) including the new *Pediococcus* species close to *P. cellicola* strains from a distilled-spirit-fermenting cellar (Zhang et al., 2005) showed high DNA base compositions.

Acknowledgments

We would like to thank Ms. Nitaya Boontim, Institute for Science and Technology Research and Development, Chiang Mai University, Chiang Mai 50200 for her helpful sending of the *miang* samples. Thanks are also due to Dr. Kazuo Komagata, Professor of the Emeritus of the University of Tokyo, Tokyo, Japan for his reviewing the manuscript; and Dr. Jean Euzéby, Professor of Laboratoire de Bactériologie, École Nationale Vétérinaire, Toulouse cedex 03, France for the etymology.

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