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Molecular identification, L-lactic acid production, and antibacterial activity of *Bacillus* strains isolated from soils

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ABSTRACT

Fourteen spore-forming bacteria isolated from soils were evaluated for their taxonomic characterization, lactic acid production, and antimicrobial activity. They were belonged to the genus *Bacillus* and were closely related to *Bacillus coagulans* LMG 6326^T with 97.48%–98.48% similarity, based on 16S rRNA gene analyses. Repetitive genomic element-PCR (REP-PCR) fingerprinting using the primers sets; BOX-PCR, ERIC-PCR, GTG_5-PCR, and REP-PCR were used to differentiate among the species. Clustering of the isolates with the PCR fingerprint dendrograms obtained two groups. Group 1 consists of two isolates, JC-3 and JC-11 (14.29%), and the rest of isolates (85.71%) were distributed in another group. Based on 16S rRNA sequence analysis and REP-PCR and the phenotypic characteristics, they were classified as a novel *Bacillus* species. These isolates were screened for lactic acid production and antimicrobial efficiency, and the results revealed that they produced L-lactic acid in the range of 1.7 ± 0.1 g/l–32.6 ± 0.7 g/l at 98.58% ± 0.06%–100.00% ± 0.00% optical purity. Among them, only JC-19 was found to show inhibitory activity against *Kocuria rhizophila* ATCC 9341.

INTRODUCTION

Bacillus species are the largest members of the Family *Bacillaceae* in Phylum Firmicutes. They are Gram-positive aerobic or facultative anaerobic endospore-forming rods. More than 200 *Bacillus* species are widely distributed in nature but are predominant in soils with diverse phenotypic and phylogenetic characteristics (Vos *et al*., 2011). *Bacillus* strains have a great potential to use in various applications as enzymes producers, probiotics, bioprotection products, vitamins, and antibiotics, meanwhile, some strains cause food poisoning and serious infection. They play critical roles in the biodegradation of pollutants in the environment (Feto, 2016). A spore-forming lactic acid bacteria, *Bacillus coagulans* that shares characteristics of both genera *Lactobacillus* and *Bacillus* are marketed probiotics (earlier known as *Lactobacillus sporogenes*) which widely used as a microbial supplement for improving digestive health and nutrient utilization (Liao and Nyachoti, 2017). They also used as an alternative to antibiotics in animal feed and have a beneficial role in improving products quality in farming industries, especially in pig and poultry production (Ezema, 2013). From their numerous characteristics, it was hard to distinguish within the species (De Clerck *et al*., 2004). To overcome the conventional identification based on morphological, physiological and biochemical characteristics, several molecular techniques have been developed using polymerase chain reaction (PCR) consisting to amplification of 16S ribosomal DNA sequences with broad-range primers for rapid identification (Jensen *et al*., 1993). This PCR technique provided a rapid and accurate description of species, which has led to large changes in bacterial taxonomy. Several PCR techniques (RT-PCR and Rep-PCR) were developed for detection and species differentiation of *Bacillus* group (Da Silva and Valicente, 2013; Oliwa-Stasiak *et al*., 2011). Repetitive sequence-based PCR (Rep-PCR) method is useful to differentiate microbes by using specific primers which have been designed to match the conserved repetitive sequences distributed in bacterial genomes. The amplification product of DNA fragments consisting of sequences between the repetitive elements at different sizes

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can be fractionated by electrophoresis, and the resulting DNA fingerprint patterns can be compared to analyze the genetic relationship between strains. Identification of *Bacillus* group using Rep-PCR showed high specific results at low cost (Rampadarath *et al*., 2015).

The present study was undertaken to isolate, identify, and to compare strains of *Bacillus* isolated from soil by using a combination of morphological, physiological, biochemical characteristics and 16S rRNA sequence analysis. The genetic similarity of strains was characterized using BOX-PCR, ERIC-PCR, GTG_5-PCR, and REP-PCR. Lactic acid production and antimicrobial ability were also investigated.

MATERIALS AND METHODS

Sources and isolation methods

A total of 14 bacterial strains were isolated from soil samples collected from Nakornnayok Province and Bangkok, Thailand (Table 1) by enrichment approach using Glucose-Yeast extract-Peptone (GYP) broth which composed of (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) for 3 days at 37°C under anaerobic conditions (Prasirtsak *et al*., 2013). The aliquot was streaked on GYP agar plate containing 0.5% $CaCO_3$ and the single colony was picked up and purified on GYP agar plates for further study.

Identification methods

Phenotypic characterization

The isolates were identified based on their phenotypic characteristics, including morphological characteristic on GYP agar plate containing $CaCO_3$ after incubated under anaerobic conditions at 37°C for 2 days. Gram staining, spore formation, catalase activity, nitrate reduction, arginine, starch and gelatin hydrolysis were determined (Cowan and Steel, 2004; Tanasupawat *et al*., 1998) . The effects of NaCl tolerance in GYP broth supplemented with 0%, 3%, 5%, 7%, 9%, and 10% (w/v) NaCl, temperature for growth at 15°C, 20°C, 30°C, 37°C, 45°C, 50°C, 55°C, 60°C, 65°C and pH growth at pH 4.5–9.0 (0.5 interval) were performed. Acid from carbohydrates was tested as previously described (Tanasupawat *et al*., 1998).

Genotypic characterization

Genomic DNA extractions of the isolates were extracted using the *AccuPrep*™ Genomic DNA Extraction Kit (Bioneer, Daejeon, Republic of Korea) and the quality and quantity of DNA were measured to determine acceptable purity using Nanodrop ND-2000 UV-Vis spectrophotometer (Thermo Scientific, Wilmington, DE). Repetitive sequence based on polymerase chain reaction (REP-PCR) fingerprinting with BOX-PCR, ERIC-PCR, GTG_5-PCR, and REP-PCR was conducted to obtain the genomic fingerprinting of all bacterial isolates. The oligonucleotide primers used in this study were: for BOX-PCR, BOX-A1R: 5′-CTACGGCAAGGCGACGCTGACG-3′, for ERIC-PCR, ERIC-1R: 5′-ATGTAAGCTCCTGGGGATTCAC-3′ and ERIC-2: 5′-AAGTAAGTGACTGGGGTGAGCG-3′, for GTG5-PCR: 5′-GTGGTGGTGGTGGTG-3′, and REP-PCR: REP1R-I, 5′-IIIICGICGICATCIGGC-3′. Optimal PCR program for each of the primer sets used was described by Versalovic *et al*. (1994). Amplification was done in 25 µl reaction mixture consisting 100 ng/µl of template DNA, 10 × PCR reaction buffer containing 20 mM $MgCl_2$, 20 pmol/µl each of the primers, 2.5 mM of the dNTPs mixture, and 2 U/µl of Takara *Taq* DNA polymerase (Takara Bio Inc, Japan). PCR amplification was performed in a Bio-Rad T100 PCR thermal cycler (Bio-Rad Laboratories, Hercules, CA). The BOX-PCR consisted of an initial denaturation step at 97°C for 7 minutes, which was followed by 30 cycles of 94°C for 1 minute, 53°C for 1 minute, 56°C for 4 minutes, and final extension at 65°C for 16 minutes. For ERIC-PCR and GTG_5-PCR, the conditions were 95°C for 5 minutes, which was followed by 30 cycles of 94°C for 0.45 minute, 40°C for 1 minute, 65°C for 10 minutes, and final extension at 65°C for 20 minutes. The amplification of REP-PCR was performed as follows: 95°C for 5 minutes, which was followed by 45 cycles of 94°C for 0.45 minute, 40°C for 1 minute, 65°C for 10 minutes, and final extension for 20 minutes at 65°C. The PCR amplification products (10 µl) were subsequently visualized by electrophoresis in a 1% (w/v) agarose gels (15 ×

Table 1. Location of soil samples, isolate number, 16S rRNA gene sequence similarity (%), and nearest relative.

Province	Isolate no.	Similarity (%)	Nearest relative
Nakornnayok	PP-13	98.08	*B. coagulans* LMG 6326^T
Nakornnayok	PP-18	98.19	*B. coagulans* LMG 6326^T
Bangkok	JC-1	97.62	*B. coagulans* LMG 6326^T
Bangkok	JC-3	98.07	*B. coagulans* LMG 6326^T
Bangkok	JC-4	98.21	*B. coagulans* LMG 6326^T
Bangkok	JC-5	98.18	*B. coagulans* LMG 6326^T
Bangkok	JC-6	98.17	*B. coagulans* LMG 6326^T
Bangkok	JC-7	97.64	*B. coagulans* LMG 6326^T
Bangkok	JC-8	97.48	*B. coagulans* LMG 6326^T
Bangkok	JC-11	98.08	*B. coagulans* LMG 6326^T
Bangkok	JC-12	97.60	*B. coagulans* LMG 6326^T
Bangkok	JC-17	97.71	*B. coagulans* LMG 6326^T
Bangkok	JC-19	98.21	*B. coagulans* LMG 6326^T
Bangkok	JC-20	98.48	*B. coagulans* LMG 6326^T

25 cm) for 2.20 hours at a constant voltage of 150 volts in 0.5× Tris-borate-Ethylenediaminetetraacetic acid (EDTA) buffer at room temperature (25°C). The gel was stained with ethidium bromide (EtBr 0.5 µg/ml), then the digital image capturing was visualized under UV light using Gel document™ XR^+ imaging system (Bio-Rad, USA) and the image was printed. The DNA fingerprint patterns were analyzed by using the software package, GelCompar II version 5.10 (Applied Maths, Kortrijk, Belgium). The similarity among digitized pattern was calculated using Pearson correlation coefficient, and the unweighted pair-group method with average linkages (UPGMA) dendrogram was derived from the profiles. A clustering level of 80% was regarded as significant grouping (Gevers *et al*., 2001). The different dendrograms were visually interpreted to set the delineation level separately for each species. For the identification of strains, the 16S rRNA gene was determined as described below.

The gene encoding 16S rRNA was amplified by PCR with the 16S bacteria specific 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) forward and 518F (5′-CCAGCAGCCGCGGTAATACG-3′) reverse primer. Agarose gel electrophoresis was performed to validate the quality of the PCR product. Purified PCR fragments were sequenced with the both primers and more six primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′),785F (5′-GGATTAGATACCCTGGTA-3′), 518F (5′-CCAGC AGCCGCGGTAATACG-3′), 800R (5′-TACCAGGGTATCTA-ATCC-3′), 907R (5′-CCGTCAATTCMTTTRAGTTT-3′), and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′). The amplified 16S rRNA gene sequence was analyzed by Macrogen®, Korea. In the phylogenetic analysis, a homology search was carried out using the Basic Local Alignment Search Tool of Nucleotide (BLASTN) search program at the NCBI Website. Sequence gaps and ambiguous nucleotides were removed prior to the construction of phylogenetic trees. Phylogenetic analysis was carried out using neighbor-joining methods (Saitou and Nei, 1987) and was constructed by using the program MEGA7 (Tamura *et al*., 2013). The confidence value of individual branches was determined with bootstrap values based on 1,000 replications (Felsenstein, 1985). 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

Quantification of lactic acids produced by the isolates was performed. All isolates were transferred to GYP agar slant and incubated at 37°C for 48 hours under aerobic condition. Cell grew on the slant was inoculated into 50 ml of seed medium consisting of (per liter 10 g glucose,15 g yeast extract, 4 g NH_4Cl, 0.50 g KH_2PO_4, 0.50 g K_2HPO_4, 5 g $CaCO_3$, and 20 ml salts solution) and incubated at 37°C, 200 rpm for 5 hours. Each 25 ml pre-culture broth was transferred into 25 ml glucose solution (240 g/l) containing 4 g $CaCO_3$. They were incubated at 37°C. Samples were collected at 72 hours after that the supernatant was acidified, centrifuged, and diluted to the proper concentration to analyze lactic acid and residual glucose by high-performance liquid chromatography (HPLC) equipped with Biorad, Aminex HPX-87H ion exclusion organic acid column (300 mm × 7.8 mm) 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/minute (Shimadzu-LC-10 Avp). A refractive index detector (Shimadzu-RID-10A) was used

Table 2. Differential phenotypic characteristics of the isolates.

Characteristics	Isolate no.															
	PP-13	PP-18	JC-1	JC-3	JC-4	JC-5	JC-6	JC-7	JC-8	JC-11	JC-12	JC-17	JC-19	JC-20	LMG 6326^T	KCTC 13078^T
Growth temp (°C)	30–55	30–55	30–55	30–55	30–60	30–55	30–55	30–55	30–60	30–55	30–60	30–60	30–60	30–55	25–60	20–55
Max. NaCl (%)	0	0	0	0	0	0	0	0	3	0	0	3	0	0	3	3
MR	−	+	−	−	−	−	−	−	−	+	−	−	−	−−	−	−
VP	+	+	+	−	−	+	+	+	+	+	−	−	+	−	+	+
Aesculin	−	+	−	−	−	−	−	−	+	−	−	+	−	+	−	−
Acid from																
L-Arabinose	+	+	−	−	+	+	+	+	−	−	+	+	+	+	−	+
D- Cellobiose	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	−
Lactose	+	+	+	+	+	+	−	−	−	+	+	+	+	w	+	−
D-Mannitol	+	+	−	+	−	+	+	+	+	+	+	+	+	−	+	+
D-Mannose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
D-Melibiose	+	+	−	+	+	+	−	+	−	+	+	−	+	+	+	−
Methyl-α-D-glucoside	+	−	−	−	−	+	+	+	−	−	+	+	+	+	w	+
Raffinose	+	+	w	w	+	+	+	+	−	w	+	w	+	+	w	−
Rhamnose	+	+	−	−	−	+	+	+	−	−	+	w	+	−	−	+
D-Ribose	−	−	+	+	+	+	+	w	+	+	w	w	+	w	−	+
Sucrose	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	−
D-Trehalose	+	+	+	+	+	+	+	+	+	+	+	w	+	+	+	−
D-Xylose	+	−	−	−	+	+	+	+	w	+	+	w	w	w	−	+

+ = positive reaction; w = weak reaction; − = negative reaction.

to detect the organic compounds detail. Lactic concentration and their isomers were analyzed by high-performance liquid chromatography (Prasirtsak *et al*., 2013).

Screening of antimicrobial activity

All bacterial isolates were investigated to evaluate their antibacterial activity against tested microorganisms, including three strains of Gram-positive bacteria, *Bacillus subtilis* ATCC 6633, *Kocuria rhizophila* ATCC 9341, and *Staphylococcus aureus* ATCC 6538, two strains of Gram-negative bacteria, *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922, and one of yeast strain, *Candida albicans* ATCC 10231 by using agar diffusion method (Acar and Goldstein, 1991). The tested bacteria were cultivated on nutrient agar plate (NA, Difco) at 37°C and yeast on Sabouraud Dextrose Agar plate (SDA, Difco) at 30°C, for 24 hours. A new agar plate surface was inoculated by spreading each microorganism and 50 µl of culture broth of each isolate was introduced into a 6 mm diameter hole. The plates were incubated at the same condition. The inhibition zones (mm) were observed and measured.

RESULTS AND DISCUSSION

Isolation and phenotypic characterization

Fourteen strains of bacteria consisted of JC-1, JC-3, JC-4, JC-5, JC-6, JC-7, JC-8, JC-11, JC-12, JC-17, JC-19, and JC-20 were isolated from the soil in Bangkok, except PP-13 and PP-18 that were isolated from soils in Nakornnayok province (Table 1). Colonies of isolates were circular, smooth, shiny, and white and approximately 1 mm in diameter after incubation on GYP agar plate at 37°C for 2 days. All isolates were catalase positive, oxidase negative, and facultative anaerobe. Methyl red, Voges–Proskauer, and aesculin reactions were variable among each isolate. They grew at 30–55 to 60°C, at pH 5–8 and some could grow at 3% NaCl. Acid was produced from D-fructose, D-galactose, D-glucose, D-maltose, and some isolates produced acid from L-arabinose, D-cellobiose, lactose, dextran, inulin, D-mannitol, D-mannose, D-melibiose, methyl-α-D-glucoside, raffinose, rhamnose, D-ribose, sucrose, D-trehalose, and D-xylose as shown in Table 2.

Genotypic characterization

A total of 14 strains and two type strains, *B. coagulans* LMG 6326^T and *B. acidiproducens* KCTC 13078^T were subjected to PCR fingerprinting. The results of numerical analysis of the generated PCR banding patterns are shown in a dendrogram (Figs. 1–4). The fourth set of primers, BOX-PCR, ERIC-PCR, GTG_5-PCR, and REP-PCR, gave successful amplification with band size lying between 500–5,000 bp, 50–10,000 bp, 200–15,000 bp, and 100–8,000 bp, respectively. In the present study, the REP-PCR technique with a set of primers, BOX-PCR, GTG_5-PCR, and REP-PCR, similarly revealed considerable diversity between isolates, generating a similarity dendrogram with four clusters. The fingerprint of isolates assessed presented unique patterns distinguished from the pattern of type strains, with low similarity, indicating that they were derived from distinct strains. About 90% similarity, indicating similar strains, was observed between 10 of the isolates. Clusters with this similarity were found between the set of primers (BOX-PCR, GTG_5-PCR, and REP-PCR) and ERIC-PCR. This might be the ERIC primer suitable for the family *Enterobacteriaceae* than the *Bacillus* group. Based on these findings, it can be concluded that the use of REP-PCR fingerprinting with the primers BOX-PCR, GTG_5-PCR, and REP-PCR is beneficial, easy-to-perform, with a single-performance protocol and these approaches have shown to be useful for assessing the genetic diversity of *Bacillus* strains. Moreover, the consumables and labor cost of these analyses method are significantly lower than those for other genotyping methods (Duan *et al*., 2009; Olive and Bean, 1999) and also promising genotypic tool for rapid and reliable speciation and grouping of *Bacillus* species.

Phylogenetic analysis was used to study the relationships of bacteria by comparison of 16S rRNA gene sequences with GenBank sequence database using BLAST software. The 16S rRNA gene sequence of the isolates exhibited 97.48%–98.48% similarity to the sequences of *B. coagulans* LMG 6326^T in NCBI database (Fig. 5). In addition, their phenotypic characteristics

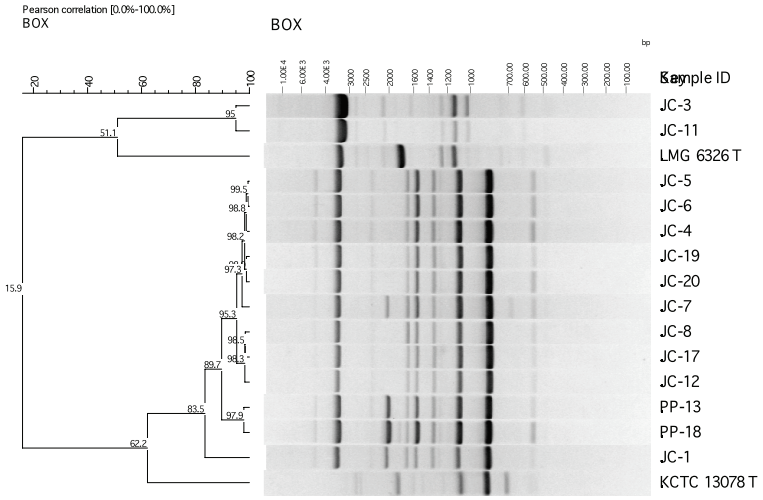

Figure 1. The dendrogram of BOX-PCR fingerprints patterns of isolates, *B. coagulans* LMG 6326^T and *B. acidiproducens* KCTC 13078^T based on UPGMA cluster analysis of Pearson correlation coefficient values.

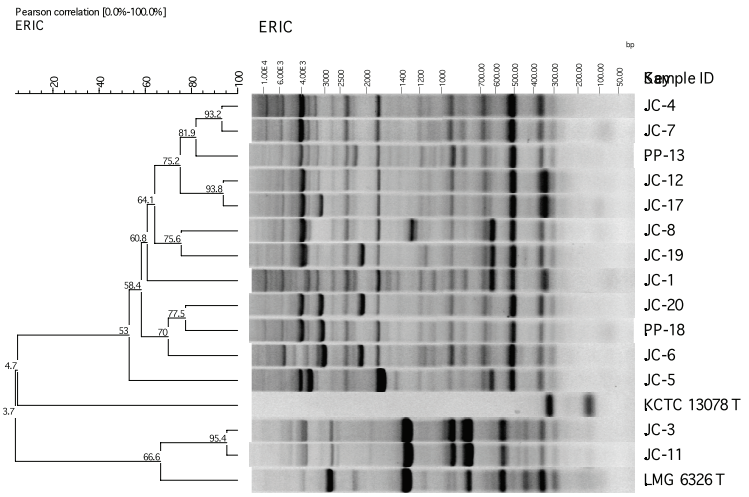

Figure 2. The dendrogram of ERIC-PCR fingerprints patterns of isolates, *B. coagulans* LMG 6326^{T} and *B. acidiproducens* KCTC 13078^{T} based on UPGMA cluster analysis of Pearson correlation coefficient values.

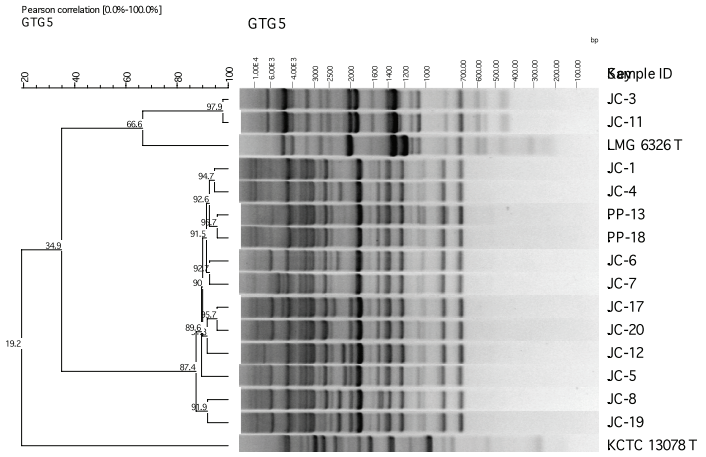

Figure 3. The dendrogram of GTG$_5$-PCR fingerprints patterns of isolates, *B. coagulans* LMG 6326^{T} and *B. acidiproducens* KCTC 13078^{T} based on UPGMA cluster analysis of Pearson correlation coefficient values.

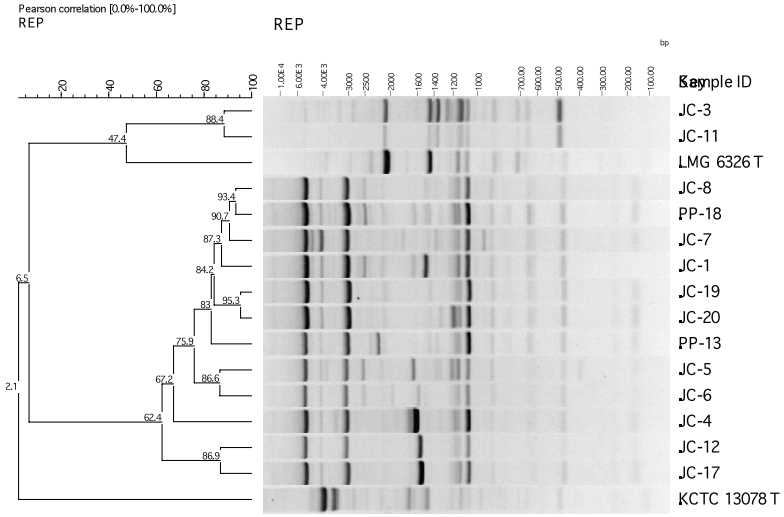

Figure 4. The dendrogram of REP-PCR fingerprints patterns of isolates, *B. coagulans* LMG 6326^{T} and *B. acidiproducens* KCTC 13078^{T} based on UPGMA cluster analysis of Pearson correlation coefficient values.

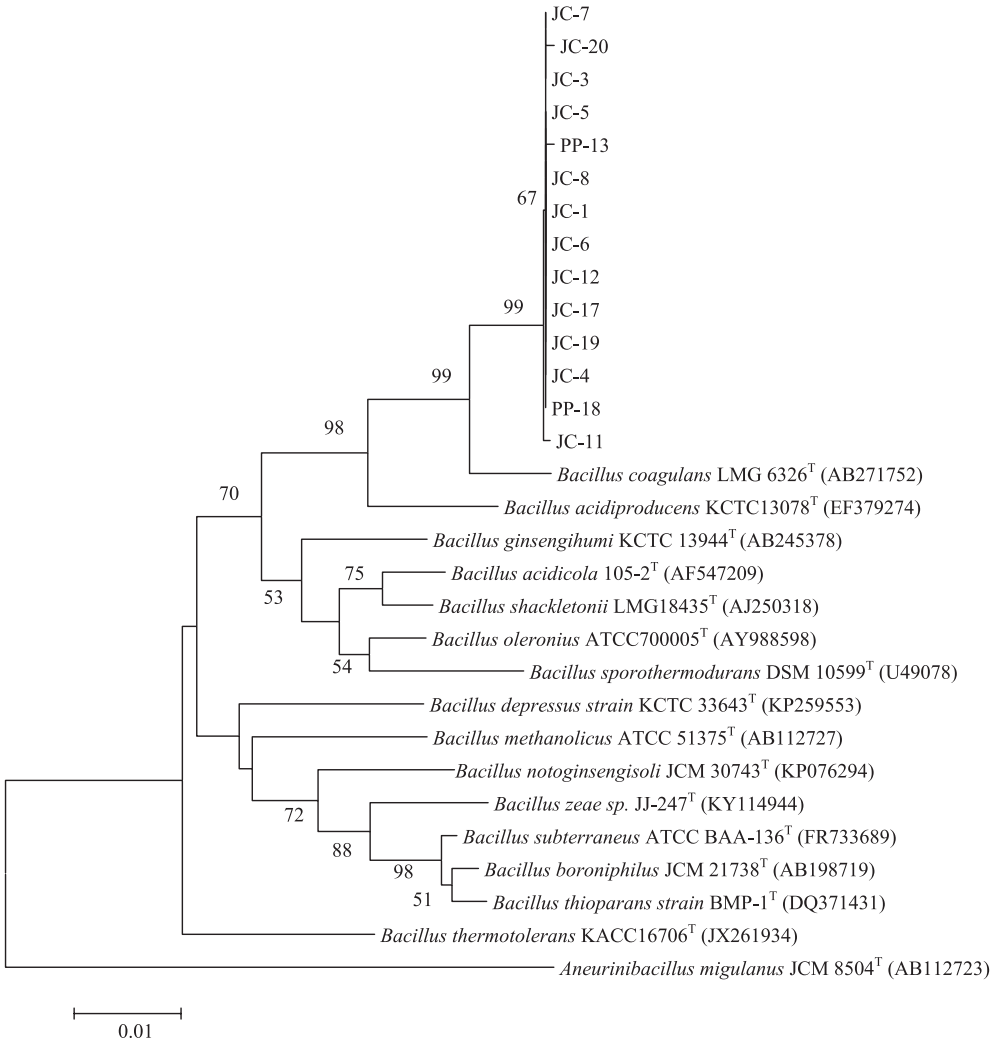

Figure 5. Phylogenetic tree constructed by the neighbour-joining method showing the position of isolates and related *Bacillus* species based on 16S rRNA gene sequences.

of the isolates showed some differences from *B. coagulans* LMG 6326^T. However, there were some variable characteristics as in Table 2. Therefore, all the isolates were the novel species of the genus *Bacillus* based on 16S rRNA gene sequences (De Clerck *et al*., 2004; Logan *et al*., 2009) and the further taxonomic characterization is required.

Lactic acid production

All isolates were catalase positive so they were tested for lactic acid production under aerobic condition. The results on the total lactic acid production, yield, productivity, and qualitative isomer of L-and D-lactic acid by using HPLC of each strain were shown in Table 3. The isolates produce L-lactic acid in the low range (1.7 ± 0.1 g/l–32.6 ± 0.7g/l) at 98.58% ± 0.06%–100.00% ± 0.00% optical purity which is far different to the efficiency of the close related type strains, *B. coagulans* and *B. acidiproducens*. At the same condition, *B. coagulans* LMG 6326^T and *B. acidiproducens* KCTC 13078^T could produce L-lactic acid at 66.7 ± 2.8 g/l and 82.8 ± 1.0 g/l, respectively. Strain JC-7 showed the highest L-lactic acid production efficiency at 32.6 ± 0.4 g/l with 100.00 ± 0.00 optical purity. However, these new species could produce L-lactic acid less than the *Bacillus* strains as previously reported (Ma *et al*., 2014; Ohara *et al*., 1996; Thitiprasert *et al*., 2017).

Antimicrobial activity

The result revealed that only *Bacillus* sp. JC-19 was potentially effective in suppressing the bacterial growth of *K. rhizophila* ATCC 9341. It was also reported antibacterial activities against pathogenic bacteria by *B. coagulans* CGMCC 9551 isolated from healthy piglet feces (Gu *et al*., 2015).

CONCLUSION

In this study, 14 isolates from soil in Thailand were identified as novel *Bacillus* species based on their physiological and biochemical properties as well as its 16S rRNA genes. With the primers (BOX-PCR, GTG_5-PCR, and REP-PCR), they showed similar four clusters on dendrogram that belonging to the same species. It showed that some strains, JC-3 and JC-

Table 3. Lactic acid and residual glucose of isolates.

Isolate no.	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)			
PP-13	1.7 ± 0.1	0.67 ± 0.04	0.02 ± 0.00	120.00	117.45 ± 0.07	100.00 ± 0.00
PP-18	12.2 ± 2.2	0.30 ± 0.07	0.17 ± 0.03	120.00	79.75 ± 2.47	100.00 ± 0.00
JC-1	27.9 ± 2.6	0.50 ± 0.02	0.38 ± 0.04	120.00	65.17 ± 0.92	100.00 ± 0.00
JC-3	1.8 ± 0.0	0.74 ± 0.06	0.03 ± 0.00	120.00	117.55 ± 0.21	100.00 ± 0.00
JC-4	12.2 ± 1.6	0.48 ± 0.02	0.17 ± 0.02	120.00	94.65 ± 4.6	100.00 ± 0.00
JC-5	17.0 ± 0.2	0.56 ± 0.01	0.24 ± 0.00	120.00	89.60 ± 0.85	100.00 ± 0.00
JC-6	2.5 ± 0.1	0.60 ± 0.12	0.03 ± 0.00	120.00	115.85 ± 0.92	100.00 ± 0.00
JC-7	32.6 ± 0.4	0.53 ± 0.02	0.45 ± 0.01	120.00	58.65 ± 1.2	100.00 ± 0.00
JC-8	11.6 ± 0.0	0.40 ± 0.01	0.16 ± 0.00	120.00	91.1 ± 0.42	98.58 ± 0.06
JC-11	28.2 ± 0.8	0.56 ± 0.04	039 ± 0.01	120.00	69.8 ± 1.98	100.00 ± 0.00
JC-12	2.4 ± 0.3	0.28 ± 0.05	0.03 ± 0.00	120.00	111.1 ± 2.69	100.00 ± 0.00
JC-17	25.0 ± 1.1	0.62 ± 0.01	0.35 ± 0.02	120.00	79.45 ± 1.06	100.00 ± 0.00
JC-19	28.3 ± 3.6	0.63 ± 0.05	0.39 ± 0.00	120.00	74.87 ± 0.57	100.00 ± 0.00
JC-20	31.8 ± 2.3	0.82 ± 0.04	0.44 ± 0.03	120.00	81.25 ± 4.6	100.00 ± 0.00
LMG 6326^T	66.7 ± 2.8	0.70 ± 0.02	0.93 ± 0.04	120.00	24.45 ± 1.2	100.00 ± 0.00
KCTC 13078^T	82.8 ± 1.0	0.69 ± 0.01	1.15 ± 0.01	120.00	0.00 ± 0.00	99.68 ± 0.24

11, showed highly similar patterns using ERIC primer. The results indicated the potential of REP-PCR technique as a tool for examining relationships among our *Bacillus* species. For lactic acid production efficiency, the isolate JC-7 showed the highest L-lactic acid production efficiency at 32.6 ± 0.4 g/l with 100.00 ± 0.00 optical purity. In addition, only one isolate, JC-19 showed the antimicrobial activity against *K. rhizophila* ATCC 9341.

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CONFLICT OF INTERESTS

There are no conflicts of interest.

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