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Characterization of lactic acid-producing *Bacillus coagulans* strains with their antibacterial activity and L-lactic acid production

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ABSTRACT

Objectives: The objective is to study the screening of antibacterial activity, identification, and L-lactic acid production of spore-forming *Bacillus* isolates. **Materials and Methods:** Fourteen *Bacillus* isolates were characterized based on their phenotypic characteristics and 16S rRNA gene sequence analyses. The antibacterial activity was determined by agar diffusion method, and lactic acid production was analyzed using a high-performance liquid chromatography. Four isolates that produced above 85.0 g/L of lactic acid were selected for lactic acid fermentation using mid-log seed culture. **Results:** All isolates were identified as *Bacillus coagulans* based on the phenotypic characteristics and 16S rRNA gene sequence similarity (99.02–99.78 %). Strain JC-15 and JC-16 exhibited antibacterial activity against *Kocuria rhizophila* ATCC 9341. All isolates produced L-lactic acid ranged from 56.7 ± 0.54 to 92.05 ± 0.23 g/L at 37°C under microaerophilic conditions with 99.12 ± 0.10–100 ± 0.00% optical purity of lactic acid. *B. coagulans* PP-16 showed highest L-lactic acid concentration at 92.05 ± 0.23 g/L, yield of 0.77 ± 0.00 g/g, and productivity of 0.96 ± 0.00 g/L.h. After controlled seed preparation conditions, the four selected strains produced L-lactic acid in the range of 93.25 ± 0.07–103.25 ± 0.21 g/L and 102.85 ± 0.49–111.85 ± 0.21 g/L at 37°C with 98.75 ± 0.00–99.70 ± 0.01 and 88.66 ± 0.02–99.60 ± 0.01% optical purity of lactic acid under microaerophilic and anaerobic conditions, respectively. Yield and productivity were also increased. Fermentation under anaerobic conditions showed higher L-lactic acid titers and yield, whereas microaerophilic conditions gave higher productivity. Strain PP-16 gave the highest optical purity in both conditions.

Keywords: Antibacterial activity, *Bacillus,* L-lactic acid, soil

INTRODUCTION

Bacillus species were Gram-positive endospore-forming rods, include halophilic, thermophilic, psychrophilic, acidophilic, and alkalophilic bacteria. They were distributed in diverse habitats including soil, compost, water and intestinal tracts.[1,2] *Bacillus* species exhibit a wide range of physiological characteristics and play an important roles in biotechnological applications for the production of various valuable products such as enzymes, vitamins, and antimicrobial compounds.[3,4] *Bacillus subtilis, B. coagulans B. clausii*, *B. Pumilus,* and *B. cereus* strains were listed as safe on the Food and Drug Administrations generally regarded as safe and used as probiotics in the Food and Pharmaceutical Industries.[4,5] The probiotic *B. coagulans* is a lactic acid-producing, spore-forming bacterial species that could survive under the acidic environment of the stomach and the intestine due to the formation of spores. *B. coagulans* probiotic strains that proved as safe were used as alternative antibiotics. They produced antimicrobial compounds to inhibit enteric pathogen,

promoting immune system, and growth by improving digestion and absorption of nutrients in the intestinal tract.[6,7] In addition, *B. coagulans* strains were thermophilic bacteria that produce high optically pure L-lactic acid which essential for the polymerization of high-grade polylactic acid.[8,9] Lactic acid is also conventionally used as a natural preservative in the Food Pharmaceuticals and Cosmetics. Increasing demand of lactic acid monomer for the synthesis of polylactic acid biodegradable polymers with applications in medical, pharmaceutical drug, and food industries expanded its market.[10] In this study, This study deals with the screening of antibacterial activity, lactic acid production and taxonomic characterization of the isolates. Furthermore, we investigated the effect of seed culture to L-lactic acid production for more efficient fermentation.

MATERIALS AND METHODS

Sources and Isolation Methods

Bacterial strains were isolated from soil samples collected from various areas in Nakornayok and Bangkok, Thailand [Table 1]. Approximately 0.25 g of each soils was enriched in 5 mL glucose yeast extract and peptone (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 100 mL solution) and incubated under anaerobic conditions at 37°C for 3 days. About 40 μL aliquot of enriched medium was spread on GYP agar plate containing 0.5 % $CaCO_3$ and incubated at the same temperature for 2 days. They were reisolated until pure cultures were obtained and kept for further study as described by Prasirtsak *et al.*[11]

Identification Methods

Phenotypic characterization

Morphological characteristics were determined on the cells cultivated on GYP agar plate containing $CaCO_3$ after incubated under anaerobic conditions at 37°C for 2 days. Catalase activity, methyl red (MR), and Voges–Proskauer (VP) test, aesculin hydrolysis, nitrate reduction, arginine hydrolysis, urease and oxidase, growth in 0, 3, 5, 7, 9, and 10% (w/v) NaCl concentrations, and growth at temperature 15, 20, 30, 37, 45, 50, 55, 60, and 65°C and at pH 4.5–9.0 (0.5 interval) were performed. Acid formation from various carbohydrates was carried out as previously described by Tanasupawat *et al*.[12]

Genotypic Characterization

The 16S rRNA gene was PCR amplified using primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 518F (5'CCAGCAGCCG CGGTAATACG'3). Agarose gel electrophoresis was performed to validate the quality of the PCR product. The amplified 16S rRNA gene sequence was analyzed by Macrogen®, Korea. The sequences of strains were aligned with selected sequences obtained from GenBank using CLUSTAL_X version 1.81. The alignment was edited manually to remove gaps and ambiguous nucleotides before the construction of phylogenetic trees. A phylogenetic tree was constructed by the neighbor-joining method[13] with the program MEGA7.[14] The confidence values of individual branches in the phylogenetic tree were determined using the bootstrap analysis based on 1000 replications.[15] The values for sequence similarity among the closest strains were determined using the EzTaxon server.[16]

Screening of Antimicrobial Activity

Antibacterial activity was tested by agar diffusion method. All isolates were cultured in nutrient broth at 37°C for 48 h. The strains of *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *B. subtilis* ATCC 6633, *Kocuria rhizophila* ATCC 9341, *Staphylococcus aureus* ATCC 6538, and *Candida albicans* ATCC 10231 were used as tested microorganisms. All tested microorganisms were cultivated in NA agar plate (NA, Difco) at 37°C for 24 h. A new NA agar plate surface is inoculated by spreading each tested microorganism over the entire agar surface. Then, a hole with a diameter

Table 1: Isolate number, location of soil, 16S rRNA gene sequence similarity (%), and identification

Isolate no.	Province	% Similarity*	Accession number	Identification
PP-11	Nakornnayok	99.55	LC337948	*B. coagulans*
PP-12	Nakornnayok	99.47	LC337949	*B. coagulans*
PP-14	Nakornnayok	99.62	LC337950	*B. coagulans*
PP-15	Nakornnayok	99.17	LC337951	*B. coagulans*
PP-16	Nakornnayok	99.77	LC337952	*B. coagulans*
PP-17	Nakornnayok	99.09	LC337953	*B. coagulans*
JC-2	Bangkok	99.7	LC337954	*B. coagulans*
JC-9	Bangkok	99.57	LC337955	*B. coagulans*
JC-10	Bangkok	99.62	LC337956	*B. coagulans*
JC-13	Bangkok	99.78	LC337957	*B. coagulans*
JC-14	Bangkok	99.65	LC337958	*B. coagulans*
JC-15	Bangkok	99.67	LC376037	*B. coagulans*
JC-16	Bangkok	99.18	LC337959	*B. coagulans*
JC-18	Bangkok	99.02	LC337960	*B. coagulans*

*Similarity with *B. coagulans* ATCC 7050^T. *Bacillus coagulans: B. coagulans*

of 6 mm is punched aseptically with a sterile cork borer and 50 μL of culture broth of each isolates was introduced into the well. The agar plates were incubated at 37°C for 48 h. The antimicrobial producing microorganisms exhibited inhibitory growth against tested microorganisms. The inhibition growth zones (mm) were measured and recorded.

First Screening of Lactic Acid Production

All isolates were transferred to the new GYP agar slant and incubated at 37°C for 48 h. Cells grew on the slant was inoculated into 50 ml of preculture 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 h. Each 25 mL preculture broth was transferred into 25 mL glucose solution (240 g/L) containing 4 g $CaCO_3$. Fermentations were carried out in 250 mL Erlenmeyer flasks at 37°C under microaerophilic condition for 72 h with shaking at 150 rpm. The microaerophilic condition was performed by plugging of the flasks with T-type silicone stopper. Cell growth was determined based on turbidity at 600 nm. Total lactic acid, their isomers, other end products, and residual glucose were determined by high-performance liquid chromatography (HPLC).

Effects of Seed Culture Age on Lactic Acid Production

The four isolates such as PP-14, PP-16, JC-2, and JC-18 were all catalase positive. They were cultivated aerobically on GYP agar for 48 h. 0.5 mL of bacterial suspension of each isolate (optical density at 600 nm of 30–40) was inoculated into preculture medium (GYP broth) and incubated at 37°C (200 rpm) under aerobic condition.[17] The aerobic condition was performed by plugging of the flasks with C-type silicone stopper for good air permeability. Cell growth and residual glucose were determined. Seed inoculum at the mid-log phase of the four isolates cultivated at 37°C (200 rpm) was prepared and each of 25 mL seed culture broth was added into 25 mL glucose solution (240 g/L) containing 4 g $CaCO_3$ as a neutralizing agent. Fermentations were carried out at initial glucose concentration of 120 g/L in 250 mL Erlenmeyer flasks at 37°C under microaerophilic and anaerobic conditions with shaking at 150 rpm. Anaerobe was performed by putting the flask in a W-zip pouch containing AnaeroPack-Anaero (Mitsubishi gas chemicals Inc., Japan). Samples were taken every 12 h for the analysis of cell growth, total lactic acid, and residual glucose.

Determination of Lactic Acid and End Product

At the end of fermentation, the supernatant was taken and acidify with 1M HCl. The concentrations of lactic acid and the residual glucose were determined by HPLC equipped with Aminex HPX-87H ion exclusion organic acid column (Bio-Rad, USA) 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. Lactic concentration and their isomers were analyzed by HPLC.[11]

RESULTS AND DISCUSSION

Isolation and Identification

Fourteen isolates such as PP-11, PP-12, PP-14, PP-15, PP-16, PP-17, JC-2, JC-9, JC-10, JC-13, JC-14, JC-15, JC-16, and JC-18 are Gram-positive endospore-forming rods. Colonies were cream, convex with entire margin (1-3 mm in diameter) on GYP agar medium. They were all facultative anaerobic, MR, and catalase positive but negative for aesculin hydrolysis, indole formation, nitrate reduction, and urease. They grew at 20–60°C, at pH 5.0–8.5, and in the range of 0–9 % NaCl. Acid is produced from D-fructose, D-galactose, D-glucose, D-maltose, D-mannitol, D-melibiose, and D-trehalose. Variable characteristics were found on VP test, arginine hydrolysis, and acid production from L-arabinose, D-cellobiose, lactose, D-mannose, methyl-α-D-glucoside, raffinose, rhamnose, D-ribose, sucrose, and D-xylose. Phylogenetic analysis based on 16S rRNA gene sequence revealed that the isolates were closely related to *B. coagulans* ATCC 7050^T with 99.02-99.78% similarity [Table 1 and Figure 1]. The phenotypic characteristics of some isolates showed variable characteristics compared to *B. coagulans* ATCC 7050^T as in Table 2 and as previously described by. Nakamura *et al*. and De Clerck *et al*.[18,19] Therefore, they were identified as *B. coagulans*[18,19] based on the phenotypic characteristics and 16S rRNA gene similarity.

Antibacterial Activity

Bacillus coagulans JC-15 and JC-16 showed antibacterial activity against *K. rhizophila* ATCC 9341 (formerly known as *Micrococcus luteus*), Gram-positive cocci bacteria in the family *Micrococcaceae*. *K. rhizophila* formerly considered as non-pathogenic bacteria until some reported in the late 20th century that showed its infection potential in humans causing central catheter damage, methylmalonic aciduria, and acute cholecystitis in patients.[20] *K. rhizophila* is widely used as a standard quality control strain for antimicrobial susceptibility and sterility testing in the food industry. Otherwise, it was also reported that *B. coagulans* CGMCC 9551 isolated from healthy piglet feces showed wide range of antibacterial activities against pathogenic bacteria and had potential probiotic characteristics.[21]

First Screening of Lactic Acid Production

All isolates were first screened for lactic acid production ability at 37°C under microaerophilic condition. The results on the total lactic acid production and qualitative isomer of L-and D-lactic acid using HPLC are shown in Table 3. They produced final L-lactic acid ranging from 56.7 ± 0.54 to 92.05 ± 0.23 g/L with 99.12 ± 0.1–100 ± 0.00% optical purity. Isolate PP-16 gave the highest lactic acid titer at 92.05 ± 0.23 g/L and productivity of 0.96 ± 0.00 g/L.h. with 100 ± 0.00% optical purity. Our isolates showed high potential for L-lactic acid production with high optical purity that prospered for biodegradable plastic polymerization. Michelson *et al*. reported that *B. coagulans* SIM-7 produced L-lactic acid at 91.5 g/L and productivity of 1.28 g/L.h. in

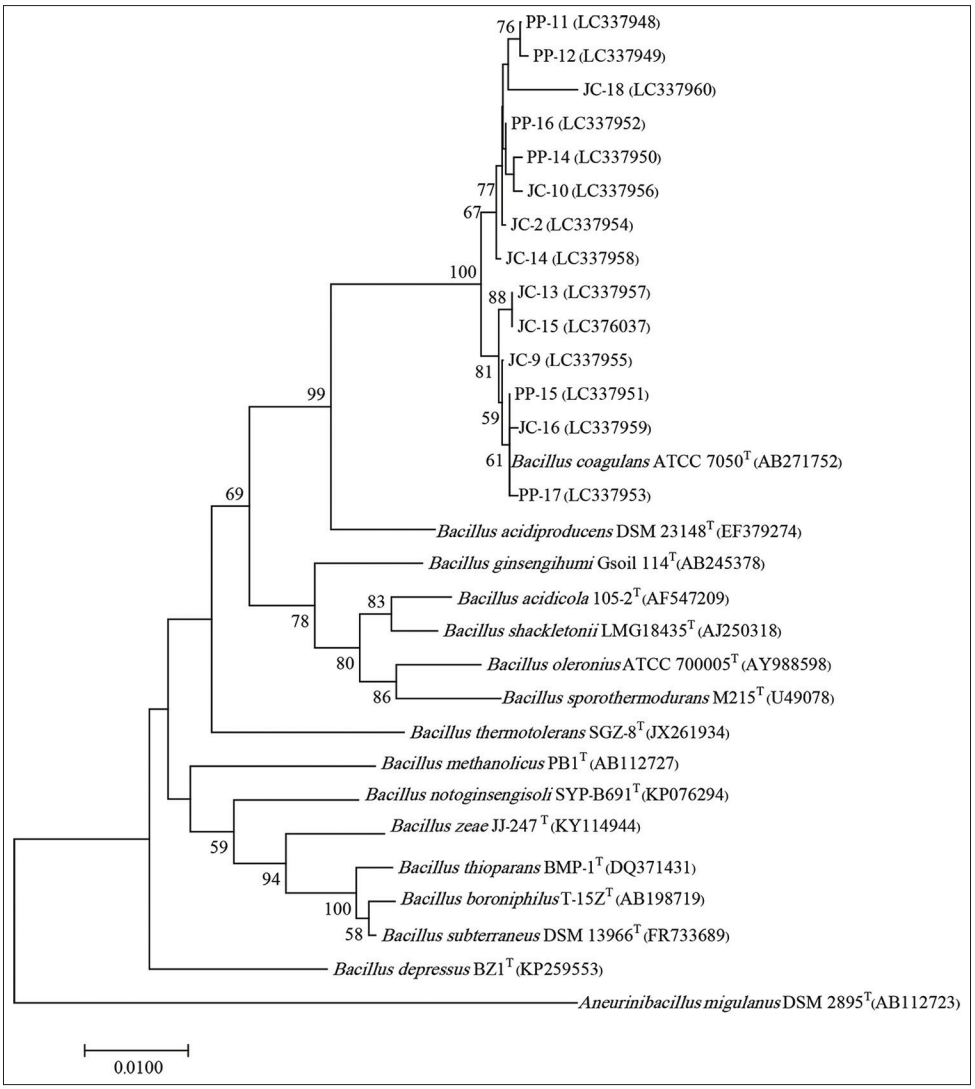

Figure 1: Phylogenetic tree constructed using the neighbor-joining method showing the position of *Bacillus coagulans* isolates based on 16S rRNA gene sequences

batch cultivations.[22] It was reported that *Bacillus* sp. strain 2-6 isolated from soil produced 118.0 g/L of L-lactic acid at 97.3% yield, 4.37 g/L/h productivity with an optical purity of 99.4% in batch fermentation.[23] Zhou *et al.*[24] also reported that *B. coagulans* WCP10-4 isolated from soil could produce optically pure L-lactic acid from 240 g/L glucose and starch giving 210 g/L of L-lactic acid and a productivity of 3.5 g/L/h in batch fermentation. From 14 isolates, of which four isolates (PP-14, PP-16, JC-2, and JC-18) produced L-lactic acid above 85 g/L were chosen for further study.

Effect of Seed Culture Age and Fermentation Conditions on Lactic Acid Production

Growth profile of the four selected isolates was investigated (data not shown) and seed culture at log phase was used for lactic acid fermentation studies. *B. coagulans* PP-14, PP-16, JC-2, and JC-18 were investigated either in microaerophilic or anaerobic conditions for the production of lactic acid. Controlling of seed culture age has a positive effect on lactic acid fermentation as shown in Table 4. L-Lactic acid produced, yield (Yp/s), and productivity were increased compared to the former experiments (Table 3). The isolates produced L-lactic acid in the range of 93.25 ± 0.07–103.25 ± 0.21 g/L and 102.85 ± 0.49–111.85 ± 0.21g/L at 37°C with 98.75 ± 0.00–99.70 ± 0.01 and 88.66 ± 0.02–99.60 ± 0.01% optical purity under microaerophilic and anaerobic, respectively. One-way analysis of variance was run to compare the lactic acid titers and productivity of the four strains in both conditions. It was shown that strain PP-14 and JC-2 gave the highest L-lactic acid titer in microaerophilic and anaerobe conditions, respectively, with statistically significant at $P \leq 0.05$. The results showed that the production of lactic acid of the four strains could occur under microaerophilic and anaerobe conditions. Fermentation under anaerobic showed higher L-lactic acid titer and yield, whereas microaerophilic conditions gave higher productivity and % optical purity except strain PP-14 that showed higher L-lactic acid concentration, yield, and productivity under anaerobic than that obtained from microaerophilic conditions. Oxygen played an important role on growth and showed negative effect on lactic acid fermentation by *B. coagulans*.[9,25]

Table 2: Differential characteristics of isolates and *B. coagulans* ACTC 7050^T

Characteristics	Isolate number														ACTC 7050^T
	PP-11	PP-12	PP-14	PP-15	PP-16	PP-17	JC-2	JC-9	JC-10	JC-13	JC-14	JC-15	JC-16	JC-18	
Growth temperature (°C)	30–55	25–55	30–60	25–60	20–60	20–60	20–60	25–55	25–55	25–60	25–60	25–60	30–60	20–60	20–60
Growth in NaCl (%)	0–3	0	0–3	0–3	0–9	0–3	0–5	0–3	0	0–3	0–3	0–3	0–5	0–7	0–3
Growth at pH	5–8	5–8	5–8	5–8	5–8	5–8	5–8	5–8	5–8.5	6–8	5–7	5–8	5–8	5–8	5–8
VP	+	+	-	-	-	+	+	-	+	+	-	-	+	+	+
Arginine hydrolysis	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-
Oxidase	+	+	+	+	+	+	-	-	+	+	+	-	-	+	+
Acid form															
L-Arabinose	+	+	+	+	+	-	+	+	+	-	+	+	+	+	-
D-Cellobiose	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
Lactose	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
D-Mannose	+	+	+	+	-	+	-	-	-	+	-	+	-	-	+
Methyl-α-D-glucoside	-	-	-	+	+	+	+	+	+	+	+	+	+	+	w
Raffinose	+	+	+	+	-	-	+	-	+	-	+	-	-	+	w
Rhamnose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
D-Ribose	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sucrose	-	-	-	+	-	-	-	-	-	+	-	-	-	-	+
D-Xylose	-	-	+	+	+	+	-	-	+	-	+	-	-	+	-

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

Table 3: Lactic acid production of isolates under microaerophilic conditions

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-11	82.71±0.52	0.74±0.00	0.86±0.01	120.00	8.80±0.21	100±0.00
PP-12	72.11±0.49	0.74±0.00	0.75±0.01	120.00	22.10±0.42	100±0.00
PP-14	88.70±0.74	0.74±0.01	0.92±0.01	120.00	0.00±0.00	99.17±0.08
PP-15	76.60±0.18	0.73±0.01	0.80±0.00	120.00	14.80±0.54	100±0.00
PP-16	92.05±0.23	0.77±0.00	0.96±0.00	120.00	0.00±0.00	100±0.00
PP-17	67.60±0.58	0.68±0.01	0.70±0.01	120.00	20.90±0.57	100±0.00
JC-2	86.40±0.83	0.75±0.01	0.93±0.01	120.00	0.00±0.00	100±0.00
JC-9	70.40±0.38	0.74±0.01	0.73±0.00	120.00	24.95±0.35	100±0.00
JC-10	73.50±0.52	0.74±0.01	0.77±0.01	120.00	21.00±0.42	99.12±0.10
JC-13	63.91±0.37	0.74±0.00	0.67±0.00	120.00	34.20±0.42	99.17±0.01
JC-14	56.70±0.54	0.69±0.01	0.59±0.01	120.00	38.05±0.49	100±0.00
JC-15	75.90±0.31	0.76±0.01	0.79±0.00	120.00	19.70±0.47	100±0.00
JC-16	74.01±0.50	0.77±0.01	0.77±0.01	120.00	23.40±0.60	100±0.00
JC-18	87.71±0.19	0.78±0.01	0.91±0.00	120.00	8.01±0.16	99.39±0.00
B. coagulans ATCC 7050^T	68.60±0.51	0.71±0.01	0.71±0.00	120.00	23.60±0.47	100±0.00

Bacillus coagulans: B. coagulans

B. coagulans is catalase positive. In the presence of oxygen, they generated energy from glucose through glycolysis, citric acid cycle (TCA), and the electron transport chain which resulted in high growth rate. Lactic acid was produced with high productivity from higher cell mass.[9,17] For anaerobic fermentation, the metabolic flux shifted toward the anaerobic fermentation route leading to lower cell mass, and glucose was consumed for lactic acid production so higher yield were achieved. Ohara and Yahata[26] reported that *Bacillus* sp. SHO-1, *B. cereus*, *B coagulans*, *B. subtilis*, and *B. thuringiensis* gave

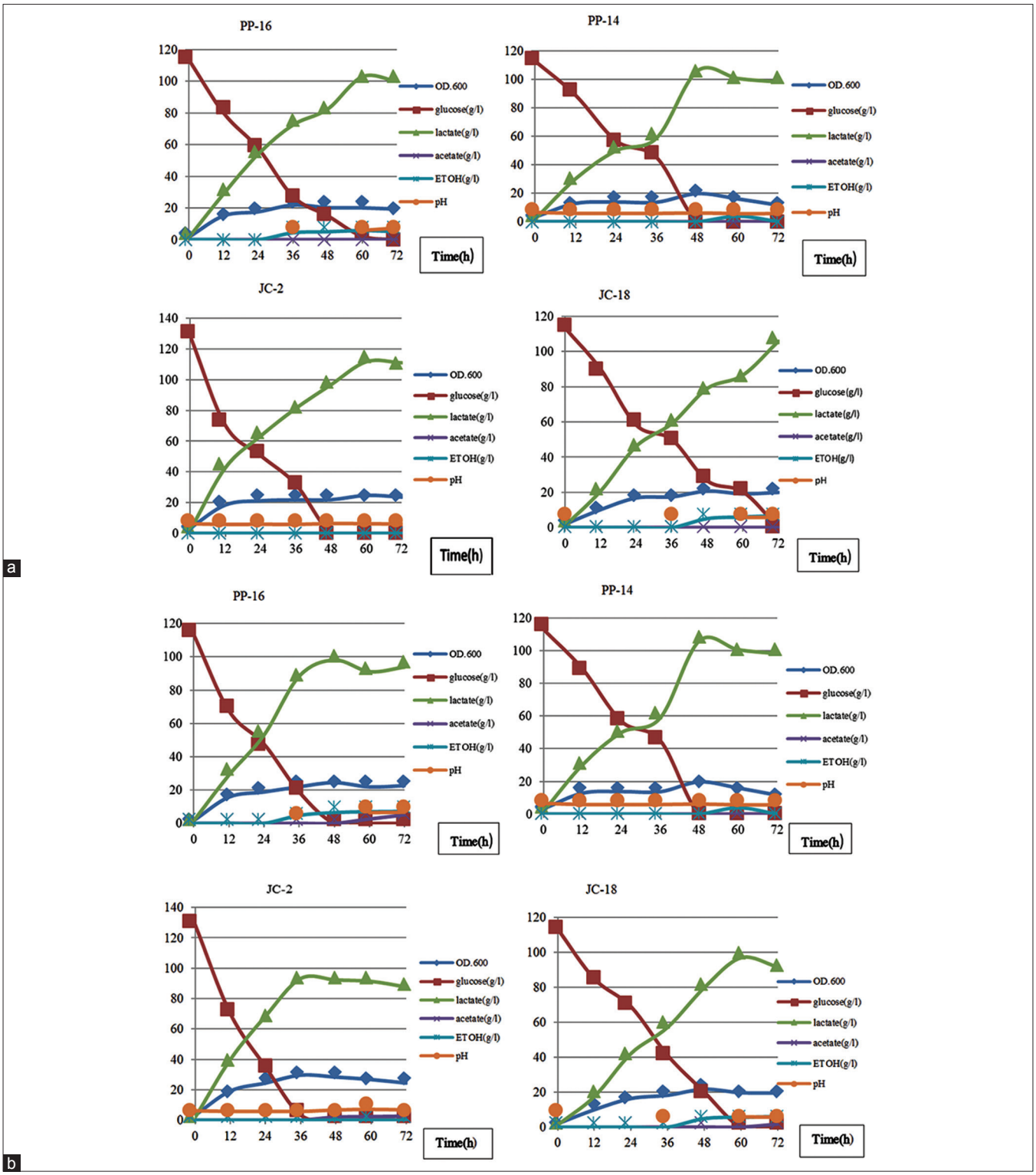

Figure 2: Time courses of lactic acid production, cell growth, residual glucose, and other end products of selected isolates. (a) anaerobic fermentation, (b) microaerophilic fermentation

more lactic acid titer in anaerobic culture, but higher yield was obtained in the aerobic culture. It was also reported that *Terrilactibacillus laevilacticus* SK5-6, a catalase positive strain, produced high D-lactate titer, yield, and productivity under anaerobic conditions.[27] For our study, microaerophilic fermentation also gave higher cell growth and productivity than anaerobic. It was observed that L-lactic acid produced was associated to cell growth, after depletion of glucose to zero and maximum L-lactic acid concentration was obtained, acetate and ethanol were appeared, and L-lactic acid concentration was decreased either in microaerophilic or anaerobe conditions [Figure 2].

Table 4: Lactic acid production (g/L) of selected isolates with controlled seed condition under microaerophilic (M) and anaerobic conditions (An)

Parameter	Isolate PP-14		Isolate PP-16		Isolate JC-2		Isolate JC-18	
	M	An	M	An	M	An	M	An
Cultivation time (h)	48	48	48	60	36	60	60	72
Maximun L-lacic acid (g/L)	103.25±0.21	106.00±0.28	98.15±0.07	102.85±0.49	93.25±0.07	111.85±0.21	96.85±0.35	105.9±0.28
Optical purity (%)	98.75±0.00	88.66±0.02	99.70±0.01	99.60±0.01	99.04±0.00	98.99±0.00	99.16±0.00	98.92±0.00
Glucose remained (g/L)	0.00±0.00	0.00±0.00	0.00±0.00	2.2±0.14	3.35±0.07	0.00±0.00	0.00±0.00	0.00±0.00
Acetic acid (g/L)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.71±0.06	0.00±0.00
Ethanol (g/L)	0.00±0.00	0.00±0.00	6.49±0.11	5.8±0.08	0.00±0.00	0.00±0.00	6.04±0.20	6.87±0.04
pH	6.17	6.15	5.84	6.49	6.33	6.74	5.7	5.85
Productivity (g/L.h)	2.15±0.00	2.20±0.01	2.04±0.00	1.71±0.01	2.59±0.01	1.86±0.00	1.61±0.01	1.47±0.00
Yield (g/g)	0.91±0.00	0.94±0.00	0.87±0.00	0.91±0.01	0.74±0.00	0.87±0.00	0.86±0.00	0.94±0.00

CONCLUSION

In this study, 14 strains isolated from soils were screened for the antimicrobial activity, lactic acid production, and identification. They possessed the ability to produce an optically pure L-lactic acid. *B. coagulans* strain JC-15 and JC-16 showed antimicrobial activity against *K. rhizophila* ATCC 9341. They produced L-lactic acid under microaerophilic condition in the range of 56.7 ± 0.54–92.05 ± 0.23 g/L at 37°C under microaerophilic conditions with 99.12 ± 0.1–100 ± 0.00% optical purity. After control seed preparation conditions, strain JC-2, JC-18, PP-14, and PP-16 could increase L-lactic acid production to 93.25 ± 0.07–103.25 ± 0.21 g/L and 102.85 ± 0.49–111.85 ± 0.21 g/L with 98.75 ± 0.00–99.70 ± 0.01 and 88.66 ± 0.02–99.60 ± 0.01% optical purity under microaerophilic and anaerobe conditions, respectively. Microaerophilic fermentation gave lower L-lactic acid and yield with high optical purity. From these studies, we concluded that the four *B. coagulans* could produce L-lactic acid both under microaerophilic and anaerobic conditions; seed quality and fermentation conditions have an effect on L-lactic acid concentration, yield (Yp/s), productivity, and optical purity. Strain PP-16 gave the highest optical pure L-lactic acid in both conditions, so it will be the potential bacteria in L-lactic acid production for biodegradable plastics.

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