Biodiversity of Lipid Producing-Bacteria Isolated from Palm Oil Industry in the South of Thailand

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Abstract

In this study, the phylogenetic diversity of lipid producing-bacteria isolated from samples collected from palm oil industry in the south of Thailand have been investigated. From 328 isolated bacteria, 56 strains were identified as potential lipid biomass producing-bacteria by using total lipids measurement. The amount of lipid yield and lipid content was ranging between 1.07-4.64 g/L and 13.30-48.59%, respectively. The identification of the selected bacterial strains was conducted by biochemical test and 16S rRNA gene sequence analysis. The study revealed that the selected bacterial strains were belonging to 20 different genera distributed among Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria division in Eubacteria and Archaea. Among the selection of isolated lipid-producing bacteria, 10 isolated bacteria were found to be prominent lipid-producers with *Bacillus subtilis* TN1 as the most potential isolate with lipid content 48.59% followed by *Arthrobacter humicola* TN2 (45.62%), *Acinetobacter baylyi* TP2 (40.78%), *Bacillus subtilis* TN5 (37.47%) and *Streptomyces glebosus* SN6 (35.78%).

Keywords : lipid-producing bacteria, palm oil industry, *Bacillus subtilis*

1. Introduction

The widespread use of petroleum-based fuels raises the pollution problems and concern about global warming [1]. The sustainability of new technologies depends not only on the capacity, but also on production of cleaner resources that ensure environmental stability. Biofuels are considered the most efficient source of alternative renewable and environmentally friendly energy [2]. This has stimulated the recent interest to develop renewable energy sources such as biodiesel. Biodiesel is made from biolipids, such as triacylglycerols with short-chain alcohol through a transesterification reaction [3]. Traditionally biodiesel is obtained from vegetable oils, animal fats, waste cooking oil and greases. However, current biodiesel production is not economically competitive with petroleum-based fuel because of the high cost of the feedstocks, which accounts for 70-75% of the total cost of biodiesel production [3]. Therefore, it is necessary to exploit a cheaper and more sustainable means for lipid production. Microbial lipids produced by oleaginous microorganisms involving yeasts, molds and algae, have been well studied because they can accumulate a large number of lipids [4]. Bio-lipids from oleaginous microorganisms also have an

advantage over vegetable oil in fatty acid composition, as these can be modified in to desired level by changing the source of nutrients or substrate [5]. Moreover, under the consideration of high food and energy requirements, the use of oleaginous microorganisms could be better option than plant derived biofuels to reduce the competition between food and bioenergy crops [2, 6]. However, the bulk lipid production from oleaginous microorganisms has been hampered by the slow growth rates and complicated regulation mechanisms of these organisms [7]. Bacteria could be an alternative fatty acid producer because they have rapid growth rates, uncomplicated to upscale and facile to control the production condition [1, 3, 8, 9, 10].

Many researchers studied about the isolation, screening and biodiversity of lipid-producing microorganism, oleaginous yeast *Cystobasidium* genus was isolated from soil rich in cellulosic waste [11], *Bacillus subtilis* HB1310 was isolated from the thin-shelled walnut [12], the oleaginous *Cladosporium* sp., *Gibberella fujikuro*, *Ochrobactrum* sp., *Plectosphaerella* sp., *Tilletiopsis albescens*, *Backusella ctenidia*, and *Davidiella tassiana* were isolated from soil samples; hot spring, permafrost, wetland, sand, lawn, saline-alkali soil, high-radiation soil and farmland in Haibei, the Tibetan Plateau [9]. However to the best of our knowledge; this is the first report that describes the biodiversity of oleaginous bacteria isolated from palm oil contaminated samples. The objective of this research was to screen and identify isolated lipid-producing bacterial from palm oil industry samples in the south of Thailand.

2. Materials and Methods

Sample collection and bacteria isolation

Soil and water samples, collected from palm oil contaminated soils and water of oil refinery industries situated in the south of Thailand; Chumphon Province (LO), Krabi Province (PP), Sutun Province (TN), Songkhla Province (PT), Suratthani Province (SN) and Trang Province (TP). The samples have been serially diluted and plated onto minimal salt medium (MSM) composed of (g/L) K_2SO_4, 1.8; KH_2PO_4, 1.2; NH_2Cl, 4.0; $MgSO_4 \cdot 7H_2O$, 0.2; NaCl, 0.1; $FeSO_4 \cdot 7H_2O$, 0.1 containing 2.0% (w/v) of palm oil by spread plate method. Plates were incubated at room temperature (30±3°C) for 7 days. Morphologically distinct colonies were re-isolated by transfer onto fresh MSM agar plates at least three times to obtain pure cultures and subsequently Gram-stained. Pure cultures of the isolates were maintained on MSM agar slants and were subcultured every 15 days [10].

Screening of lipid producing-bacteria

From the lipid producing microorganisms obtained, all of isolates were activated on nutrient broth (NB) and shaken (150 rpm) at 30°C for 24 h. A 100 µL sample of each cell culture was transferred to 5 mL of MSM containing 2.0% (w/v) of palm oil in a rotary shaker at 30°C and 150 rpm for 24 h, where triplicate samples were set up to determine biomass and lipid content [13].

Identification of selected lipid-producing bacteria

The selected strains were identified first based on morphological and physiological characteristics, 16S rRNA gene sequencing, and BLAST analysis. DNA was extracted from an overnight liquid culture by standard salting out procedure. The extracted DNA was amplified by polymerase chain reaction (PCR) using the primers UFUL and URUL as previously reported by Saimmai, et al. [10]. A PCR master mix (Cat. No. M7502; Promega, Madison, USA) was used. The cycling conditions were as follows: initial

denaturation at 95°C for 5 min; 30 cycles subsequent denaturation at 94°C for 30 s; annealing at 55°C for 30 s; extension temperature 72°C for 30 s; and final extension 72°C for 5 min). The sequencing reaction was performed by BigDye terminator sequencing kit (Perkin-Elmer Applied Biosystems, MA, USA) and analyzed by an automated sequencer ABI 310 Genetic Analyzer (Applied Biosystems). Sequence homologies were examined using BLAST version 2.2.12 of the National Center for Biotechnology Information. Multiple sequence alignments were carried out using ClustalW and a consensus neighbor-joining tree was constructed using Molecular Evolutionary Genetics Analysis (MEGA) Software v.4.0. The 16S rDNA gene sequences were submitted to GenBank with an accession number.

Analytical methods

For determination of the biomass, bacterial suspensions were centrifuged using Hettich centrifuge at 10,000g for 20 min at 4ºC and cell pellets obtained after centrifugation were dried at 105ºC overnight.

For determination of lipid production, the bacterial cells in the culture broth were collected and wash three times with deionized water. The washed cells were dried at 105ºC until their weight was constant. Total lipids in the cells were extracted according to the methods of Folch et al. [14].

3. Results

Isolation and screening of lipid producing-bacteria

In total, 328 isolated bacteria were obtained from soil, sediment and water samples collected from various palm oil mill located in the south of Thailand. Hence, 65 isolated bacteria from 5 factory samples in Chumphon Province, 34 isolated bacteria from 8 factory samples in Krabi Province, 62 isolated bacteria from two factory samples in Satun Province, 58 isolated bacteria from one factory samples in Songkhla Province, 71 isolated bacteria from 11 factory samples in Suratthani Province, and 38 isolated bacteria from three factory samples in Trang Province. Sixty six percent of the isolated bacteria (216 of 328) were Gram-negative.

Ten isolated bacteria were identified as potential lipid producing-bacteria by using total lipids measurement (Table 1). Lipid yield and lipid content of all the isolated bacteria ranged between 1.07-4.64 g/L and 13.30-48.59%, respectively. It can be categorized into 5 groups on the basis of their lipid content obtained from bacterial cells (Table 1). These are Group I with production range of < 20.00% (LO2, LO6, LO10, TN8, TN9, PP2 and PP9); Group II with production range of 20.01-25.00% g/L (LO3, LO7, LO11, PP4, PP5, PP6, PT1, PT5, PT6, SN1, SN5, SN9, TN3,TN6, TN7, TP4, TP6, and TP9); Group III with production range of 25.01-30.00% (LO8, LO9, LO12, PP1, PP7, PP8, PT2, PT3, PT4, PT7, SN2, SN4, SN8, SN10, TP1, TP3, TP5 and TP7); Group IV with production range of 30.01-40.00% (LO1, LO4, LO5, PP2, SN3, SN6, SN7, TN4, TN5 and TP8); and Group V with production range of > 40.00% (TN1, TN2 and TP2) (Table 1).

Out of these 56 lipid producing-bacteria, 10 were found to be the prominent producers of lipid whose production more than 30% with isolate TN1 as the most potential producer with lipid content of 48.59%, followed by isolate TN2 (45.62%), TP2 (40.78%), TN5 (37.47%) and SN6 (35.78%), after cultivation at 30°C, 150 rpm for 48 h. In terms of biomass yield, there are no relations between lipid yield and biomass could be observed. Biomass yield of all the isolates ranged from 4.56 to 12.25 g/L and

the highest dry cell weight could be found from isolate LO4 (12.25) followed by isolate LO3 (11.74 g/L), PT3 (11.25 g/L), TP4 (10.91 g/L) and PP8 (10.89 g/L), respectively after cultivation at 30°C, 150 rpm for 48 h**.**

Table 1 Characterization of lipid producing-bacteria isolated from palm oil contaminated samples collected from palm oil industry in the south of Thailand.

Grp.	No*	DCW (g/L)	Lipid yield (g/L)	Oil content (%, dry wt)
I	LO2	9.28±1.97	1.24±0.54	13.36±2.24
	LO6	6.25±0.94	1.21±0.31	19.36±3.64
	LO10	6.47±1.25	1.25±0.98	19.32±5.63
	TN8	5.87±1.43	1.12±0.02	19.08±4.54
	TN9	6.31±1.93	1.04±0.31	16.48±3.21
	PP3	6.25±1.07	1.07±0.21	17.12±5.96
	PP9	7.45±1.03	1.05±0.18	14.09±1.43
II	LO3	11.74±3.52	2.89±0.23	24.62±2.53
	LO7	5.74±1.58	1.28±0.25	22.30±4.21
	LO11	5.12±1.23	1.25±0.21	24.41±2.13
	PP4	5.65±0.95	1.18±0.34	20.88±6.34
	PP5	5.25±0.84	1.08±0.67	20.57±5.68
	PP6	9.47±2.57	2.24±0.94	23.65±5.94
	PT1	9.25±2.74	1.87±0.15	20.22±5.90
	PT5	5.87±2.01	1.21±0.31	20.61±2.14
	PT6	9.54±1.75	2.25±0.87	23.58±3.65
	SN1	8.65±2.31	1.87±0.56	21.62±3.57
	SN5	8.45±2.56	2.02±0.89	23.91±6.24
	SN9	8.74±2.97	1.85±0.34	21.17±3.49
	TN3	6.28±1.01	1.27±0.43	20.22±4.32
	TN6	8.58±3.57	1.90±0.54	22.14±5.61
	TN7	5.52±1.90	1.28±0.36	23.19±4.46
	TP4	10.91±2.24	2.61±1.93	23.92±5.84
	TP6	9.25±1.25	2.18±0.48	23.57±3.51
	TP9	5.32±0.87	1.26±0.43	23.41±4.21
III	LO8	7.25±1.63	2.08±0.36	28.69±3.69
	LO9	10.78±2.34	3.18±0.12	29.50±5.21
	LO12	6.24±0.82	1.81±0.35	29.01±4.54
	TP1	8.58±1.02	2.25±0.14	26.22±4.34
	TP3	5.36±1.35	1.41±0.86	26.31±3.57
	TP5	8.54±0.89	2.54±0.57	29.74±6.59
	TP7	7.65±1.37	2.02±0.38	26.41±6.43
	PT2	8.47±2.87	2.41±0.87	28.45±3.53
	PT3	11.25±1.41	2.85±1.23	25.33±5.48
	PT4	8.15±2.89	2.26±0.57	27.73±3.67
	PT7	6.89±2.17	1.76±0.36	25.54±5.65
	PP1	6.87±2.58	2.02±0.57	29.40±5.31
	PP7	4.56±0.58	1.18±0.21	25.88±4.62
	PP8	10.89±3.79	3.14±0.57	28.83±6.89
	SN2	7.25±1.57	2.10±0.73	28.97±4.69
	SN4	7.54±3.74	2.02±1.63	26.79±8.54
	SN8	8.57±3.54	2.18±0.89	25.44±5.67
	SN10	7.20±1.21	2.02±0.53	28.05±4.87
IV	LO1	8.18±2.65	2.51±0.96	30.68±4.20
	LO4	12.25±3.91	3.78±0.17	30.86±3.57
	LO5	8.47±0.85	2.57±0.24	30.34±4.05

Table 1 Characterization of lipid producing-bacteria isolated from palm oil contaminated samples collected from palm oil industry in the south of Thailand (Cont.)

Grp.	No*	DCW (g/L)	Lipid yield (g/L)	Oil content (%, dry wt)
IV	PP2	7.99±3.91	2.56±0.86	32.04±6.87
	SN3	8.57±2.98	2.58±1.59	30.11±6.87
	SN6	5.25±1.30	1.88±0.34	35.78±5.97
	SN7	5.16±2.87	1.57±0.25	30.43±4.32
	TN4	5.32±2.35	1.76±0.65	33.08±3.67
	TN5	4.75±0.36	1.78±0.32	37.47±5.32
	TP8	3.76±1.03	1.17±0.21	31.13±2.45
V	TN1	9.55±3.51	4.64±1.02	48.59±5.87
	TN2	9.49±3.63	4.33±0.97	45.62±6.90
	TP2	9.91±2.21	4.04±1.57	40.78±5.78

Values are given as means ± SD from triplicates determinations

Identification, taxonomy and phylogeny of the lipid-producing bacteria

All of the 56 selected lipid-producing bacteria present in this study were chemoheterotrophs, the morphology of colonies and cells as well as their physiological and biochemical characteristics were tested. The final identification of all selected lipid-producing bacteria was accomplished by combining the alignment results of 16S rRNA gene sequence analysis with biochemical and physiological characteristics. Their sequences were assigned with the NCBI database and the nearest 16S rRNA gene sequences relative in GenBank are shown in Table 2.

16S rRNA gene analysis of 56 lipid-producing bacteria isolated from palm oil industry in south of Thailand reveal that all of the isolated bacteria affiliation can divide to five bacterial phylogenetic groups: Actinobacteria, Archaea, Bacteroidetes, Firmicutes and Proteobacteria. The presences of 20 bacterial genera from the 56 isolated bacteria suggest that there is a wide biodiversity of lipid-producing bacteria from palm oil industry in the south of Thailand.

Table 2 Phylogenetic analysis of the lipid-producing bacteria isolated from palm oil industry in the south of Thailand.

Taxonomic position	Strain code	16S rRNA gene sequence
		Nearest relative in GenBank
Actinobacteria	TN2	*Arthrobacter humicola* (AB279890)
	LO7	*Corynebacterium aurimucosum* (AY536427)
	SN3	*Dietzia maris* Bab3-8 (KT380950)
	LO8	*Gordonia amarae* P152 (KU597097)
	TP1	*Mycobacterium avium* (AY360329)
	PP6	*Rhodococcus erythropolis* (AB429542)
	SN2	*Rhodococcus erythropolis* (AF512836)
	SN7	*Rhodococcus fascians* (KR514533)
	TP6	*Rhodococcus fascians* CF17 (NR037021)
	TN6	*Rhodococcus opacus* FCL1069 (KM461688)
	LO3	*Streptomyces coelicolor* MSIS1 (FR856603)
	SN6	*Streptomyces glebosus* (NR116221)
	PT6	*Streptomyces lividans* AS2 (LC026160)
	SN5	*Streptomyces lividans* rrnB (Y00484)
Archaea	TP3	*Halobacteriaceae archaeon* (JF293279)
	TN8	*Halobacteriaceae archaeon* (KM875612)
Bacteroidetes	LO9	*Azorhizobium doebereinerae* (AJ003237)
	SN10	*Rhodothermus marinus* PRQ-55 (AF217497)
Firmicutes	TN1	*Bacillus subtilis* HB2 (LT546428)
	PP1	*Bacillus subtilis* BCA31 (HE716900)
	LO4	*Bacillus subtilis* JM4 (AY728013)
	TN5	*Bacillus subtilis* BY-3 (KC961634)
	TN3	*Bacillus licheniformis* (NR116023)
	PT2	*Bacillus licheniformis* IITR HR-2 (FJ447354)
	TP4	*Bacillus licheniformis* (DQ993676)
	TP5	*Bacillus licheniformis* VPS50.2 (HE993550)
	PT3	*Bacillus licheniformis* 1.1.B (AM910589)
	SN1	*Bacillus licheniformis* (JQ677088)
	LO2	*Bacillus licheniformis* RBA08 (JQ780329)
	TP9	*Bacillus mycoides* BGSC1 (AM910408)
	PP8	*Bacillus mycoides* FKS9-207 (AB677940)
Proteobacteria	SN9	*Acinetobacter baylyi* ADP1(AY289925)
	TP2	*Acinetobacter baylyi* CI14 (HG796165)
	PP2	*Acinetobacter baylyi* EBB1 (HM214924)
	PT5	*Acinetobacter calcoaceticus* (EU159482)
	SN4	*Acinetobacter calcoaceticus* (NR042387)
	LO11	*Acinetobacter junii* 97380 (HE651918)
	PP7	*Agrobacterium tumefaciens* (AJ389907)
	TN4	*Agrobacterium tumefaciens* (EU239177)
	PP5	*Escherichia coli* IMD989 (LN871242)
	LO12	*Escherichia coli* SK2 (LT545680)
	TN7	*Marinobacter aquaeolei* (AJ000726)
	PT1	*Marinobacter excellens* (AY180101)
	LO5	*Marinobacter segnicrescens* (EF157832)
	PT7	*Micrococcus luteus* (LN681571)
	TP8	*Ochrobactrum anthropic* (AM114398)
	TP7	*Pseudomonas aeruginosa* (AM419153)
	PP9	*Pseudomonas aeruginosa* (FM881781)
	PP4	*Pseudomonas putida* 19 (LN610443)
	LO1	*Pseudomonas putida* Pp (KF278708)
	PT4	*Serratia marcescens* R9-8A (HQ154570)
	SN8	*Serratia proteamaculans* Ox1a (AJ279052)
	TN9	*Serratia proteamaculans* PC1 (AJ279053)
	LO6	*Vibrio furnissii* HZN-22 (KR270125)
	LO10	*Vibrio furnissii* JCM 1282 (LC050179)
	PP3	*Vibrio ponticus* RP30 (AY897206)

Strains TN2, LO7, PP5 and LO12 exhibited high similarity (99-100%) with the genus *Arthrobacter, Corynebacterium, Agrobacterium* and *Escherichia,* respectively. Strains SN3, LO8 and TP1 shown 99-100% of similarity to the genus *Dietzia, Gordonia* and *Mycobacterium.*

The remaining 7 isolated strains, PP7; TN4; TP8; PT7; LO6; LO10 and PP2 were similar to the genus *Agrobacterium*, *Ochrobactrum, Micrococcus* and *Vibrio* with sequence similarity ranging from 99-100%, respectively.

All of the isolated bacteria in this study, 5 isolated bacteria were found to be the prominent lipid-producer with isolate TN1, TN2, TP2, TN5 and SN6 belonged to *Bacillus subtilis* HB2 (LT546428), *Arthrobacter humicola* (AB279890), *Acinetobacter baylyi* CI14 (HG796165), *Bacillus subtilis* BY-3 (KC961634) and *Streptomyces glebosus* (NR116221), respectively with the 98-100%, (Fig 1).

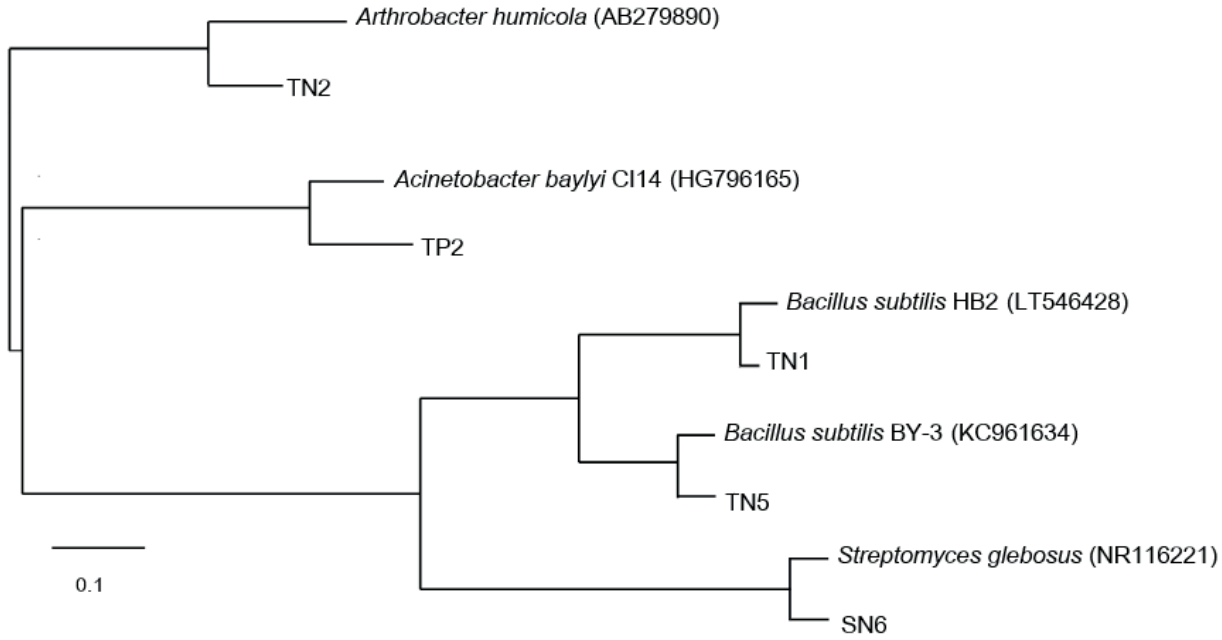

Fig. 1 Phylogenetic tree based on 16S rRNA gene sequence of five lipid-producing bacteria isolated from samples contaminated with palm oil collected from palm oil industry in the south of Thailand.

4. Discussion

Sixty-six percent of the isolated bacteria (216 of 328) were Gram-negative. It has previously been reported that most bacteria isolated from sample sites with a history of contamination by hydrocarbon or its byproducts and other immiscible substrate are Gram-negative [15, 16]. This may be a characteristic that contributes to the survival of these populations in such harsh environments [17].

The yield of oil from microorganisms depends on either species or the source of isolation [18-19]. *Bacillus subtilis* TN1 isolated from soil sample at Satun Province showed the highest lipid yield (4.64 g/L) after 48 h of cultivation. According to the previous study, it was found that the pH, N, P and K in chemical composition of the said soil sample above (Supplementary) were in the suitable range for growth of *Bacillus* sp. [20], whereas *Acinetobacter baylyi* TP2 isolated from soil sample in Trang Province and *Acinetobacter baylyi* PP2, from soil sample in Songkhla Province could

produce the lipid yield of 40.78% and 32.04%, respectively. The chemical compositions of both soil samples from Trang Province and Songkhla Province (Supplementary) were found to be similar to that of the soil sample from which the *Acinetobacter* sp. was isolated [21].

The presences of 20 bacterial genera from the 56 isolated bacteria suggest that there is a wide biodiversity of lipid-producing bacteria from palm oil industry in the south of Thailand. *Acinetobacter, Bacillus, Pseudomonas, Rhodococcus and Serratia* are the best known bacterial groups for lipid production and they were also the most commonly represented genera in our screening [4, 22]. The genus of *Bacillus*, *Acinetobacter*, *Rhodococcus*, *Pseudomonas*, *Streptomyces*, *Marinobacter* and *Serratia*, they are frequently isolated from oil-contaminated environments and many strains belonging to these genera have been demonstrated to be efficient hydrocarbon degraders and biosurfactant-producing bacteria [15, 16, 23]. *Arthrobacter, Corynebacterium, Agrobacterium* and *Escherichian* have previously been described as being able to produce lipid by using various substrates viz. acetate, citrate, fructose, glucose, glycerol, *n*-alkanes, olive oil, orange waste, succinate and valerate [22, 24]. *Dietzia, Gordonia* and *Mycobacterium* are come from actinomycetes, which biosynthesis and accumulation of lipid seems to be a common feature of bacteria belonging in this group [4, 25].

To the best of our knowledge, we are the first group to describe the genus *Agrobacterium*, *Ochrobactrum, Micrococcus* and *Vibrio* to the list of lipid-producing bacteria. All of them have been described as surface active compound-producing bacteria which difference structure such as rhamnolipids from *Ochrobactrum anthropi* 2/3 [26] and trehalose from *Micrococcus luteus* BN56 [27]. The genus *Agrobacterium* has been report as oil degradation bacteria with biosurfactant production [10]. The genus *Vibrio* has been described as EPS-producing strains which a good emulsification activity in several species such as *Vibrio harveyi* [28] *Vibrio furnissii* [29] *Vibrio campbellii* and *Vibrio fortis* [30].

5. Conclusion

In this study, 56 lipid-producing bacteria were isolated from samples contaminated with palm oil collected from palm oil industry in the south of Thailand. The phylogenetic position of all isolated bacteria was evaluated by 16S rDNA gene sequence analysis. The production of lipid was determined on isolated bacteria representative of 20 different bacterial genera distributed among Actinobacteria, Archaea, Bacteroidetes, Firmicutes and Proteobacteria. The findings of this study added 4 new genera to lipid-producing bacteria. Among them, *Bacillus subtilis* TN1, newly isolated for lipid production, produced the highest lipid content of 48.59% after cultivation at 30°C, 150 rpm for 48 h. The distribution of the selected bacterial genus divides into 20 different bacterial genera indicating that there is a wide biodiversity of lipid-producing bacteria in palm oil industry samples. Overall, the new isolated lipid-producing bacteria featured in this work display important characteristics for the future development of economically efficient industrial-scale biotechnological processes.

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Supplementary

Table S1 Properties of soil and water samples obtained from palm oil industry in the south of Thailand.

Isolate	Province	Type of sample	pH	mg/kg		
				N	P	K
LO1	Chumphon Province	Soil	6.61	21.09	15.76	30.67
LO2	Chumphon Province	Soil	6.14	18.97	11.56	49.65
LO3	Chumphon Province	Soil	7.12	15.43	30.54	76.89
LO4	Chumphon Province	Soil	7.03	22.56	8.65	36.02
LO5	Chumphon Province	Soil	6.65	30.54	21.98	78.43
LO6	Chumphon Province	Soil	6.98	23.51	19.54	56.09
LO7	Chumphon Province	Water	6.54	445.67	240.54	255.76
LO8	Chumphon Province	Soil	6.78	32.56	20.67	78.32
LO9	Chumphon Province	Soil	6.99	45.65	30.21	68.54
LO10	Chumphon Province	Water	6.42	327.56	230.09	267.54
LO11	Chumphon Province	Soil	6.87	25.04	8.87	45.33
PP1	Krabi Province	Soil	6.95	30.56	10.65	98.43
PP2	Krabi Province	Soil	7.21	26.87	12.16	101.65
PP3	Krabi Province	Water	6.75	433.58	230.54	278.44
PP4	Krabi Province	Soil	7.54	46.76	48.05	89.33
PP5	Krabi Province	Soil	7.43	40.32	51.84	95.32
PP6	Krabi Province	Soil	8.43	34.52	69.50	87.98
PP7	Krabi Province	Water	6.78	329.65	164.32	243.65
PP8	Krabi Province	Soil	7.85	30.56	10.73	104.67
PT1	Songkhla Province	Water	6.98	34.67	120.65	250.32
PT2	Songkhla Province	Soil	7.32	24.54	45.76	87.65
PT3	Songkhla Province	Soil	7.54	31.78	37.11	79.54
PT4	Songkhla Province	Water	7.22	438.43	139.06	241.67
PT5	Songkhla Province	Soil	6.89	30.43	43.76	67.89
PT6	Songkhla Province	Soil	7.01	28.54	28.54	78.21
SN1	Suratthani Province	Soil	7.54	25.76	55.76	143.67
SN2	Suratthani Province	Soil	7.21	10.54	48.21	127.98
SN3	Suratthani Province	Soil	6.87	13.78	61.87	104.67
SN4	Suratthani Province	Soil	7.41	27.95	45.89	113.66
SN5	Suratthani Province	Water	6.98	410.54	178.65	258.54
SN6	Suratthani Province	Soil	7.43	9.76	64.21	105.76
SN7	Suratthani Province	Soil	6.90	8.43	58.23	107.65
SN8	Suratthani Province	Soil	6.74	7.44	44.76	112.67
SN9	Suratthani Province	Soil	6.34	25.78	48.97	97.55
TN1	Satun Province	Soil	6.59	8.96	65.43	176.54
TN2	Satun Province	Soil	6.76	11.56	54.21	155.76
TN3	Satun Province	Soil	6.34	26.98	34.65	163.54
TN5	Satun Province	Soil	6.43	27.54	26.75	189.54
TN6	Satun Province	Water	6.78	320.56	210.54	243.76
TN7	Satun Province	Water	6.93	428.33	204.67	221.65
TP1	Trang Province	Soil	7.86	8.97	56.33	78.67
TP2	Trang Province	Soil	6.94	31.80	31.87	89.08
TP3	Trang Province	Water	5.67	311.78	198.54	237.54
TP4	Trang Province	Soil	6.87	25.76	43.54	95.12
TP5	Trang Province	Soil	7.86	32.56	12.46	87.54
TP6	Trang Province	Water	6.42	440.65	189.54	210.56
TP9	Trang Province	Soil	7.03	27.34	34.65	90.31