Vol. 18(23), pp. 494-501, 5 June, 2019
DOI: 10.5897/AJB2019.16822
Article Number: 391B4C761119
ISSN: 1684-5315

http://www.academicjournals.org/AJB

ACADEMIC JOURNALS
expand your knowledge

African Journal of Biotechnology

*Full Length Research Paper*

# Bioremediation of petroleum contaminated soils by lipopeptide producing *Bacillus subtilis* SE1

**Subuntith Nimrat[1*], Somruetai Lookchan[2], Traimat Boonthai[3] and Verapong Vuthiphandchai[4]**

[1]Department of Microbiology and Environmental Science Program, Faculty of Science, Burapha University, Chon Buri 20131, Thailand.
[2]Department of Microbiology, Faculty of Science, Burapha University, Chon Buri 20131, Thailand.
[3]Biological Science Program, Faculty of Science, Burapha University, Chon Buri 20131, Thailand.
[4]Department of Aquatic Science, Faculty of Science, Burapha University, Chon Buri 20131, Thailand.

Received 2 April 2019; Accepted 16 May, 2019

**Hydrocarbons released into ecosystems have led to environmental pollution and generated a serious threat to human health. Bioremediation is an effective method to break down hazardous hydrophobic environmental contaminants with avoiding economic and technical disadvantages. This study aimed to evaluate the efficiency of *Bacillus subtilis* SE1, a lipopeptide biosurfactant producer isolated from a petrochemical contaminated soil, on biodegradation of gasoline, diesel oil, crude oil and used engine oil in soil microcosms. During 35-day incubation, numbers of soil bacteria in petrochemical contaminated soils with *B. subtilis* SE1 addition significantly ($P < 0.05$) increased in comparison with the oil-free soils. Bioaugmentation of SE1 strain also produced a significant ($P < 0.05$) increase in percent reduction of total phenolic content in oil-polluted soils as compared to the control soils at the end of experiment. This study indicates that *B. subtilis* SE1 can be a promising hydrocarbon degrader for *in situ* bioremediation of soil environment polluted with petroleum and petrochemical products.**

**Key words:** *Bacillus subtilis*, biodegradation, bioremediation, gasoline, diesel, crude oil, engine oil.

## INTRODUCTION

Hydrocarbon pollution is currently become a critical global issue of increasing concerns regarding environmental, social and health catastrophes. Presence of different types of petroleum and petrochemical products viz. gasoline, diesel, crude oil and used engine oil, released into environments by either accidental spillage or improper disposal practices poses more aggravated problems because most disposal methods have been limited in their applications owing to expensiveness, partial effectiveness and strict environmental conditions. Gasoline constitutes mainly aliphatic hydrocarbons (41-62%) and a mixture of aromatic hydrocarbons e.g. benzene, toluene, ethylbenzene and xylene isomers (10-59%; Speight and Arjoon, 2012). Diesel oil is a refined petrochemical product composed primarily of hydrocarbon combination with carbon numbers ranging from $C_9$ to $C_{20}$, iso-alkanes, paraffinic, olefinic, naphtha and aromatic compounds as

*Corresponding author. E-mail: subunti@buu.ac.th. Tel: +66 38-103-120; Fax: +66 38-393-490.

well as trace elements of sulfur, nitrogen, metals, and oxygen (Yakimov et al., 2005). Crude oil contains thousands of different hydrophobic components like *n*-alkanes, cycloalkanes, aromatics, resins, asphaltines and heavy metals (Colwell and Walker, 1977). Polycyclic aromatic hydrocarbons (PAHs) is also a group of recalcitrant compounds found in crude oil at high percentage (Haritash and Kaushik, 2009). Used engine oil is a brown to black waste oil discharged from automobiles when oil is changed. In general, fresh engine oil comprises a higher percentage of lighter hydrocarbon and metal salts. Due to high temperature and mechanical stress during engine combustion, the oil is chemically changed by oxidation, nitration, cracking of polymers and decomposition of organometallic compounds leading to formation of other contaminants such as alkyl benzenes, naphthalenes, methylnaphthalenes and PAHs (Dominguez-Rosado and Pitchell, 2003; Lu and Kaplan, 2008). Exposure of these petroleum and petrochemicals generates a serious health risk because of some compositions known to be mutagenic and carcinogenic agents like benzene (Group 1: Carcinogenic to humans; IARC, 1987) and ethylbenzene (Group 2B: Possibly carcinogenic to humans; IARC, 2000).

In recent years, an increased attention has been paid to bioremediation by means of microbial function in a complex multiphase system, which is proposed to be more effective, environmental-friendly, and cost-effective technology (Adams et al., 2015). However, there is a limitation of biodegradation associated with poor accessibility of microorganisms to hydrophobic compounds due to their low solubility in aqueous systems compatible with microbial life (Millioli et al., 2009). This can be compensated by application of biosurfactants or biosurfactant producing bacteria in oil-polluted sites to increase the solubility and bioavailability of hydrophobic compounds and consequently, accelerate rate of biodegradation.

Among several classes of biosurfactants, lipopeptides are commonly isolated and characterized biosurfactants produced by *Bacillus* genera. This biosurfactant active compound has been reported to have several applications in cosmetics, food industry, household detergents and cleansing industries, petroleum industry, medical health and bioremediation of hydrocarbons in contaminated ecosystems (Marchant and Banat, 2012). In view point of bioremediation, lipopeptides produced by *Bacillus* species has received a great attention due to their degradation efficiency of petroleum hydrocarbon and heavy metals from contaminated soils (Bezza and Chirwa, 2015; Parthipan et al., 2017).

Recently, we isolated a biosurfactant producing strain of *Bacillus subtilis* SE1 from a waste engine oil contaminated soil in Chon Buri Province, Thailand. Biosurfactant produced by *B. subtilis* SE1 was identified as lipopeptide and found to degrade gasoline contaminated in soil biostimulated with nutrients.

Bioaugmentation of biosurfactant-producing microorganisms in hydrocarbon polluted soils is an interesting method that would generate a continuous supply of a non-toxic and biodegradable surfactant and promote the rate of biodegradation (Mnif et al., 2015). Therefore, this study was designed to evaluate *B. subtilis* SE1 ability to enhance biodegradation of gasoline, diesel oil, crude oil and used engine oil in soils under laboratory-scale bioremediation condition.

## MATERIALS AND METHODS

### Bacterial strain and culture

SE1 strain used in this study was isolated from oil-contaminated soil at a local automobile garage in Chon Buri Province, Thailand. It was proven to produce lipopeptide biosurfactant and degrade gasoline contaminated in soil. It was identified as *B. subtilis* SE1 based on its biochemical and morphological features and 16s rRNA gene sequence analysis and assigned the accession number as MH700588. *B. subtilis* SE1 was inoculated in a 250 ml Erlenmeyer flask containing 100 ml Trypticase Soy Broth (Difco, Sparks, MD, USA) in a shaking incubator (JSR, JSSI-100C, Cheongju, South Korea) at 200 rpm, 30°C for 24 h. Cell pellets were harvested by centrifuging at 8,000 rpm, 4°C for 10 min, washed thrice with sterile phosphate buffer saline (PBS). Cell pellets were re-suspended in PBS and adjusted to 1.5 A.U. at 580 nm using a spectrophotometer (Cintra 40 Double beam, GBC Scientific Equipment, Braeside, Victoria, Australia) to obtain SE1 strain number of $10^9$ CFU/ml for subsequent use.

### Biodegradation of petroleum in soil microcosm

Gasoline and diesel were purchased from a local gas station operated by Public Company Limited. Crude oil was obtained from Thai Oil Public Company Limited while used engine oil was kindly provided by an owner of local automobile garage. All petroleum products were kept in amber-colored bottles at 4°C until use. Biodegradation of petroleum and petrochemical products was tested as previously described by Abioye et al. (2012) with slight modification. A thirty-five-day long biodegradation study was set up under indoor laboratory condition in independent triplicates. Soil was collected from an automobile garage located in Chon Buri Province. Soil used in this study was loamy and dark brown in color. Soil sample (300 g) was thoroughly mixed (3 ml) with one of these petrochemicals: gasoline, diesel, crude oil and used engine oil. Each petroleum contaminated soil was divided to two batches: 1) petroleum contaminated soils and 2) petroleum contaminated soils plus *B. subtilis* SE1 suspension in a 500 ml Erlenmeyer flask. Cell suspension of *B. subtilis* SE1 (1 ml) was aspirated in the contaminated soil according to the treatment. The soil was tilled every week to maintain the moisture content and allow oxygen transfer. During static incubation at 30 °C in the dark, soils were sampled from each treatment at 2 h, 3, 7, 14, 21, 28 and 35 day post-inoculation to evaluate pH change using a calibrated pH meter (Denver instrument, UB-10, Bangkok, Thailand), viable bacteria count and total phenolic content quantification.

### Viable bacterial count

At each sampling interval, total viable cell count was evaluated using standard plating technique. Soil samples were 10-fold diluted in physiological saline prior to spreading in triplicates onto Plate Count Agar (Difco, Sparks, MD, USA). After incubation at 30°C for

24 h, all bacterial colonies were enumerated and calculated as log colony forming unit (CFU)/g.

#### Quantification of total phenolic content

Total phenolic content in the soils was assayed using Folin - Ciocalteu (FC) reagent (Box, 1983) with minor modification. Briefly, an aliquot (0.1 ml) of sample solution was mixed with 20% (w/v) $Na_2CO_3$ (1.5 ml), 10% (w/v) FC reagent (0.5 ml) and distilled water (7.9 ml) in a volumetric flask. The mixed solution was allowed to stand at room temperature in the dark. After 3 h incubation, absorbance was measured at 765 nm using a spectrophotometer. A standard curve of gallic acid was prepared and concentration of total phenolic content was quantified as gallic acid equivalent from the standard curve. Total phenolic content was calculated and expressed as percent reduction of total phenolic content (PRP) as equation below:

$$\text{PRP value (\%)} = \frac{(PC_i - PC_x)}{PC_i} \times 100$$

where $PC_i$ = initial concentration of total phenolic content and $PC_x$ = concentration of total phenolic content at day x.

#### Statistical analysis

Data were expressed as mean ± standard deviation. Data were normalized and transformed when needed. Differences were determined using a student's *t* test to determine difference between the treated and control groups at a significant level of $P < 0.05$. All statistical analyses were performed using SPSS version 19.0, Chicago, Illinois, USA.

## RESULTS

At the beginning of experiment, pH values of unbioaugmented soils were significantly ($P < 0.05$) lower than those of bioaugmented soils containing gasoline or diesel while similar pH was observed in soils with crude oil or used engine oil. At the end of experiment, pH values of petroleum contaminated soils with SE1 addition significantly ($P < 0.05$) increased ranging from 5.75 ± 0.01 to 6.03 ± 0.01, compared to those of unbioaugmented soils (5.58 ± 0.02 - 5.63 ± 0.02; Table 1).

In gasoline biodegradation study, PRP values of SE1 added soil and unbioaugmented SE1 soil increased to 14.29±2.30% and 10.44 ± 2.12%, respectively at 3 days post-incubation and remained relatively constant until 14 days post-incubation. Afterwards, PRP level of SE1 added soil significantly increased from 16.12 ± 3.16% to 27.27 ± 8.30% at 35 days post-incubation while no appreciable change occurred in unbioaugmented SE1 soil (Figure 1). A significant ($P < 0.05$) difference in PRP levels between treated and control groups was observed by 7 days post-incubation. The plate count revealed a significant ($P < 0.05$) increase in growth of soil bacteria from 5.09 ± 0.19 log CFU/g at the beginning of experiment to 7.11 ± 0.21 log CFU/g at 35 days post-incubation.

The patterns of bacterial growth and PRP value in diesel oil polluted soil were similar to those in gasoline-contaminated soil (Figure 2). A significant ($P < 0.05$) increase in bacterial count was observed since 7 days post-incubation and PRP value was significantly different ($P < 0.05$) within day 3 of experiment. PRP value of soil polluted with diesel oil with SE1 added increased sharply from 0% at the beginning of experiment to 28.24 ± 5.75% at day 3 of incubation and afterwards slowly increased until reaching 42.92 ± 4.28% at day 35 of incubation. On the contrary, PRP value of the control soil slightly increased during incubation period and reached 19.75 ± 1.35% at day 35 of incubation.

Viable bacterial counts in crude oil treated soils with/without *B. subtilis* SE1 bioaugmentation were similar in the ranges of 5.40 ± 0.03 0-5.41 ± 0.02 log CFU/g at the beginning of incubation (Figure 3). At the end of experiment, bacterial count in crude oil contaminated soil without *B. subtilis* SE1 was 6.71 ± 0.01 log CFU/g, which was significantly ($P < 0.05$) lower than that of SE1 added soil (7.16 ± 0.04 log CFU/g). Similar to diesel oil biodegradation study, a significant increase in PRP value of SE1 treated soil with crude oil contamination was noticed by 3-day post-incubation. PRP level of crude oil treated soil with *B. subtilis* SE1 addition increased obviously during incubation period until reaching 29.55 ± 4.36% at 35 days of experiment. In contrast, PRP level of crude oil contaminated soil without SE1 strain increased comparatively slowly and reached 15.06 ± 4.33% at the end of incubation.

Value of PRP in the control and treated groups related with used engine oil was relatively similar in the ranges of 0-9.95 ± 2.76% during the first 14 days of incubation. Thereafter, PRP value of soil polluted with used engine oil and added with SE1 significantly increased to 22.62 ± 3.70% at 35 day post-treatment while a slight change in PRP value occurred in soil polluted with used engine oil without SE1 added at the end of experiment (16.39 ± 1.39%; Figure 4). Viable bacterial count in used engine oil contaminated soil with SE1 addition significantly increased ($P < 0.05$) from 5.50 ± 0.05 log CFU/g at the beginning of experiment to 7.34 ± 0.03 log CFU/g at 35 days post-incubation.

## DISCUSSION

Bioaugmentation of *B. subtilis* SE1 exhibited an effective option to bioremediate the hydrocarbon polluted soils evident by significant growth of viable bacteria in the soils soaked with one of these petrochemicals: gasoline, diesel oil, crude oil and used engine oil, and increase in PRP values during a 35-day incubation. Growth of petroleum degrading bacteria in polluted soils after inoculation is important factor to facilitate biodegradation success (Das and Mukherjee, 2007). Due to *B. subtilis* SE1 isolated from soil soaked with used engine oil, it survived and adapted to grow well with hydrophobic substrates as sole carbon

**Table 1.** pH of petroleum polluted soils with or without *Bacillus subtilis* SE1 bioaugmentation for 35 days.

| Petroleum products | Beginning of experiment | | 35 days post-treatment | |
|---|---|---|---|---|
| | None (Control) | SE1 bioaugmentation | None (Control) | SE1 bioaugmentation |
| Gasoline | 5.63 ± 0.02[a] | 5.83 ± 0.03[b] | 5.59 ± 0.02[a] | 5.84 ± 0.04[b] |
| Diesel oil | 5.55 ± 0.04[a] | 5.67 ± 0.06[b] | 5.58 ± 0.02[a] | 5.75 ± 0.01[b] |
| Crude oil | 5.42 ± 0.05[a] | 5.40 ± 0.02[a] | 5.63 ± 0.02[a] | 6.03 ± 0.01[b] |
| Used engine oil | 5.37 ± 0.04[a] | 5.37 ± 0.03[a] | 5.61 ± 0.01[a] | 6.03 ± 0.01[b] |

Letters indicate significant difference ($P < 0.05$) between treatments at each sampling interval.

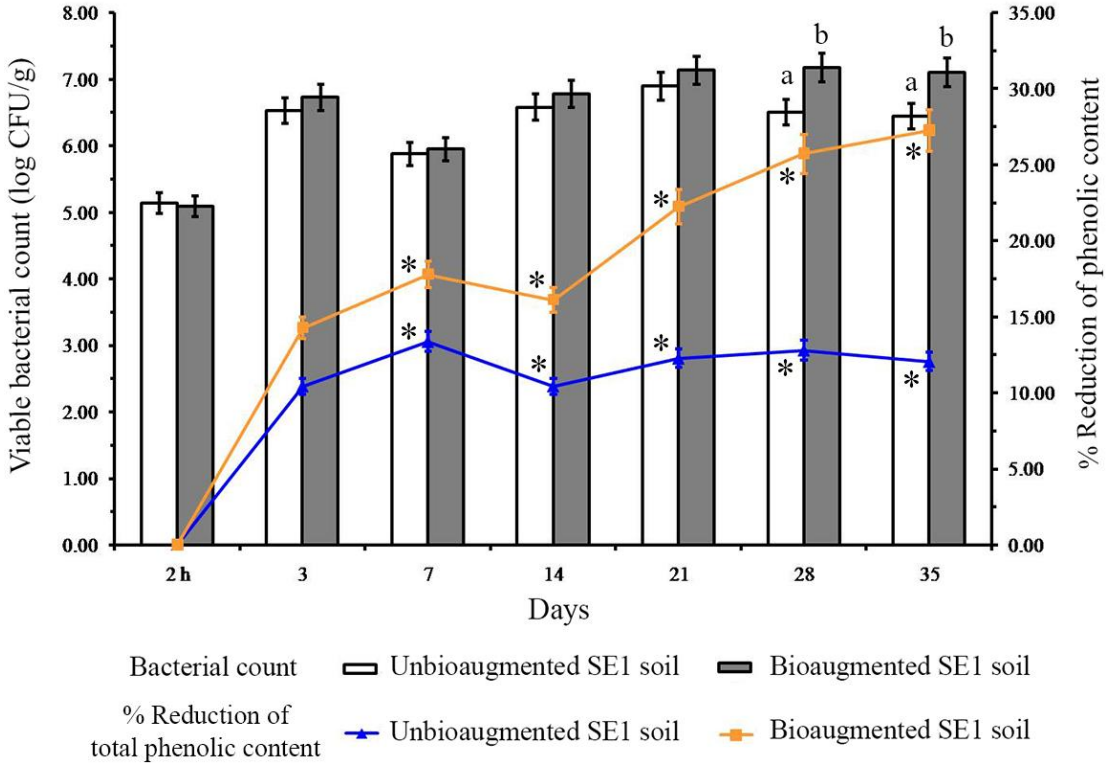


**Figure 1.** Bacterial growth and percent reduction of total phenolic content in gasoline contaminated soil during 35-day incubation. Asterisks on the lines indicate significant difference (P < 0.05) between treatments at each sampling interval. Letters on the bars indicate significant difference (P < 0.05) between treatments at each sampling interval.

source as reported by other authors (Das and Mukherjee, 2007). A significant increase in viable bacteria over the controls indicated that indigenous microflora of soil microcosms lacked ability to degrade a variety of hydrocarbons in petroleum and petrochemicals used in this study. Therefore, addition of hydrocarbon-utilizing bacteria is needed to accelerate biodegradation rate of a complex hydrocarbon mixture.

Substantial increases in PRP values were produced when inoculating *B. subtilis* SE1 into oil polluted soils. Similarly, several authors reported bioaugmentation of lipopeptide producing *Bacillus* species for biodegradation of crude oil by *B. subtilis* DM-04 (Das and Mukherjee, 2007), *B. subtilis* TB1 (Barin et al., 2014); gasoline by *B. subtilis* (Darsa et al., 2014) and diesel oil by *B. subtilis* ATCC 21322 (Whang et al., 2008). Many *Bacillus* strains were also isolated and reported to produce biosurfactant simultaneously with biodegradation of hydrocarbons in used engine oil e.g. *B. subtilis* CN2 (Bezza and Chirwa, 2015) and *Bacillus salmalaya* 139SI (Dadrasnia and Ismail, 2015). Differences in PRP values were observed when *B. subtilis* SE1 bioaugmented in soils contaminated with each type of petrochemicals. In general, biodegradation rate of petroleum hydrocarbons is dependent mainly on hydrocarbon compositions in petroleum and physicochemical characteristics of polluted systems.

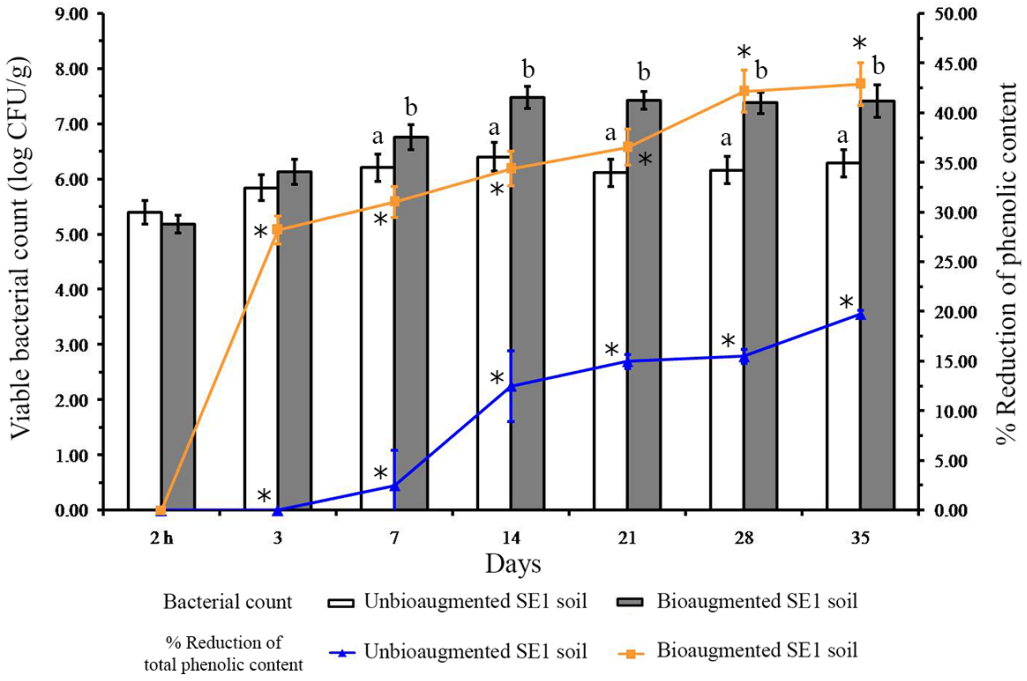


**Figure 2.** Bacterial growth and percent reduction of total phenolic content in diesel contaminated soil during a 35-day incubation. Asterisks on the lines indicate significant difference ($P < 0.05$) between treatments at each sampling interval. Letters on the bars indicate significant difference ($P < 0.05$) between treatments at each sampling interval.

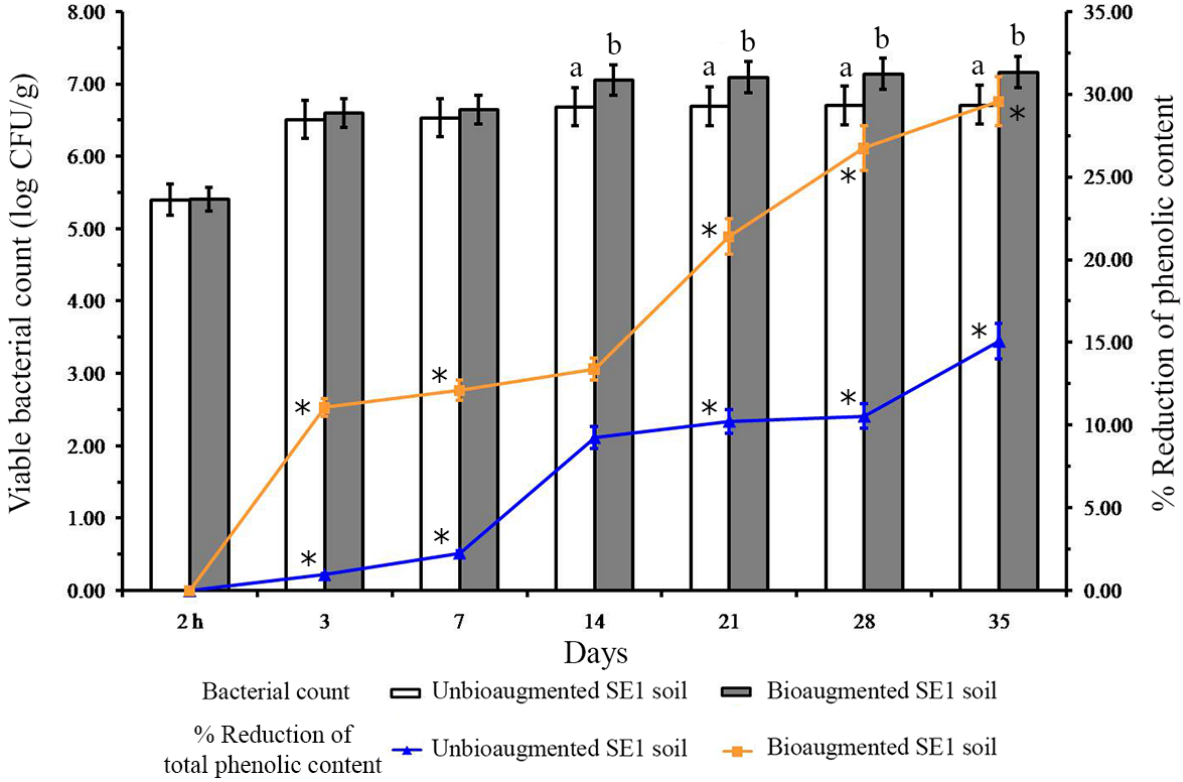


**Figure 3.** Bacterial growth and percent reduction of total phenolic content in crude oil contaminated soil during 35-day incubation. Asterisks on the lines indicate significant difference ($P < 0.05$) between treatments at each sampling interval. Letters on the bars indicate significant difference ($P < 0.05$) between treatments at each sampling interval.

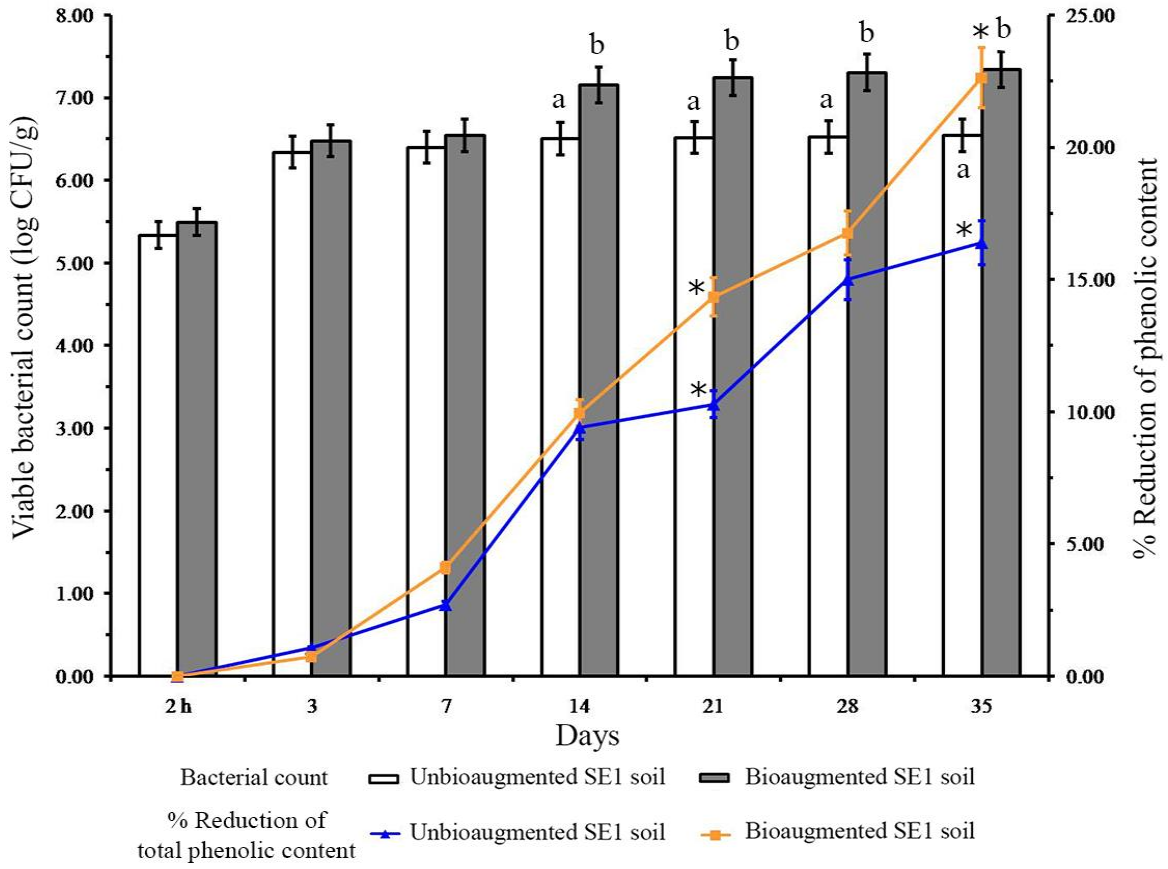


**Figure 4.** Bacterial growth and percent reduction of total phenolic content in used engine oil contaminated soil during 35-day incubation. Asterisks on the lines indicate significant difference ($P < 0.05$) between treatments at each sampling interval. Letters on the bars indicate significant difference ($P < 0.05$) between treatments at each sampling interval.

Petroleum and petrochemical products consist generally of different hydrophobic compositions. For example, diesel oil constitutes a complex mixture of hundreds of aromatic hydrocarbons, predominantly iso-alkanes, paraffinic, olefinic, naphtha and aromatic compounds (Yakimov et al., 2005) while used engine oil contains a variety of recalcitrant compounds, like alkyl benzenes, naphthalenes, methylnaphthalenes, PAHs and metals (Dominguez-Rosado and Pitchell, 2003; Lu and Kaplan, 2008). Marchut-Mikolajczyk et al. (2018) reported that *B. pumilus* 2A was an effective hydrocarbon degrader of both diesel oil and waste engine oil but exhibited different degree of diesel oil and engine oil degradation. In addition, 2A strain showed different degradation efficiency of each hydrophobic component found in the two petrochemical products.

Our recent study confirmed that bioaugmentation of *B. subtilis* SE1 together with nutrient biostimulation enhanced substantially biodegradation of gasoline contaminated in soil. In fact, supplementation of biosurfactant producing bacteria into polluted sites may provide more practical than addition of exogenous biosurfactant produced in fermentation reactors because of avoiding high cost arising from production and preparation of purified biosurfactants (Mnif et al., 2015). However, *in situ* biodegradation success of hydrocarbons depends on the selection of biosurfactant producing bacteria. In our study, all four petroleum and petrochemical products seemed to not have a harmful effect on bacterial growth. The growth of bacteria together with increase in PRP levels is possibly due to enhancing bioavailability and solubility of hydrocarbons. Biosurfactant can increase solubilization of hydrophobic compounds and enhance rate of biodegradation by two distinct ways. First, biosurfactants increase substrate bioavailability by increasing the surface area of immiscible hydrophobic substances leading to increased solubility and enhanced direct contact between bacteria and water-insoluble hydrocarbon, thereby increasing bacterial growth and rate of bioremediation. Another mechanism is associated with increased hydrophobicity of bacterial cell surfaces allowing hydrocarbon substrates to easily pass through bacterial cells (Bezza and Chirwa, 2015). In our recent study, we observed that lipopeptide produced by *B. subtilis* SE1 had high emulsifying activity and markedly reduced surface tension from 72.27 to 25.95 mN/m. Therefore, addition of *B. subtilis* SE1 with high surface tension reduction and emulsification index into hydrocarbon contaminated soils could be enough to promote the bacterial access to

hydrophobic substrates and eventually increase biodegradation of all four tested petroleum in soil systems.

Apart from increase in bioavailability of hydrophobic substrates, *B. subtilis* SE1 may produce hydrocarbon degrading enzymes resulting in improved biodegradation success in this study. It is widely known that enzymes responsible for hydrocarbon biodegradation pathways are low substrate-specific and can react with more than one hydrocarbon substrates. For example, cycloalkanes are structurally changed to their corresponding cycloalhohols or cycloketones, easily degraded by a number of bacteria in soil microcosms, by initially induced by the alkane monooxygenases (Barin et al., 2014). Therefore, presence of contributed enzymes in hydrocarbon biodegradation pathways of *B. subtilis* SE1 should be further studied.

This study encourages the application of biosurfactant-producing bacteria for *in situ* bioremediation of petroleum-contaminated environments because bioremediation process is cost-effective and environmental-friendly. Our results provided evidence that a stain of lipopeptide producer, *B. subtilis* SE1, isolated from oil-impacted soil and accustomed to environmental conditions in Thailand, enhanced significantly PRP values and promoted the growth of soil bacteria in petroleum and petrochemical polluted soils. Therefore, *B. subtilis* SE1 has a potential use for bioremediation of soil environment polluted with petroleum and petrochemical products in Thailand and may have possible applications in microbial enhanced oil recovery and related technologies.

## Conclusion

This study showed that *B. subtilis* SE1 was useful in bioremediation of soils polluted with petroleum and petrochemicals (gasoline, diesel oil, crude oil and used engine oil) because growth of soil bacteria markedly increased in petroleum contaminated soils along with significant increase in PRP levels during 35-day incubation.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENTS

This study was financially supported by the National Research Council of Thailand (grant no. 119/2560). We also appreciate the staffs of Department of Microbiology, Faculty of Science, Burapha University for providing experimental equipment and facilities.

### REFFERNCES

Abioye OP, Agamuthu P, Abdul Aziz AR (2012). Biodegradation of used motor oil in soil using organic waste amendments. Biotechnology Research International. doi: 10.1155/2012/587041

Adams GO, Fufeyin PT, Okoro SE, Ehinomen I (2015). Bioremediation, biostimulation and bioaugmention: a review. International Journal of Environmental Bioremediation and Biodegradation 3(1):28-39.

Barin R, Talebi M, Biria D, Beheshti M (2014). Fast bioremediation of petroleum-contaminated soils by a consortium of biosurfactant/bioemulsifier producing bacteria. International Journal of Environmental Science and Technology 11(6):1701-1710.

Bezza FA, Chirwa EMN (2015). Production and applications of lipopeptide biosurfactant for bioremediation and oil recovery by *Bacillus subtilis* CN2. Biochemical Engineering Journal 101:168-178.

Box JD (1983). Investigation of the folin-ciocalteau phenol reagent for the determination of polyphenolic substances in natural waters. Water Research 17(5):511-525.

Colwell RR, Walker JD (1977). Ecological aspects of microbial degradation of petroleum in the marine environments. Critical Reviews in Microbiology 5(4):423-445.

Dadrasnia A, Ismail S (2015). Biosurfactant production by *Bacillus salmalaya* for lubricating oil solubilization and biodegradation. International Journal of Environmental Research and Public Health 12(8):9848-9863.

Darsa KV, Thatheyus AJ, Ramya D (2014). Biodegradation of petroleum compound using the bacterium *Bacillus subtilis*. Science International 2(1):20-25.

Das K, Mukherjee AK (2007). Crude petroleum-oil biodegradation efficiency of Bacillus subtilis and Pseudomonas aeruginosa strains isolated from a petroleum-oil contaminated soil from North-East India. Bioresource Technology 98(7):1339-1345.

Dominguez-Rosado E, Pitchel J (2003). Chemical characterization of fresh and weathered motor oil via GC/MS, NMR and FITR Techniques. Proceedings of the Indiana Academy of Science 112(2):109-116.

Haritash AK, Kaushik CP (2009). Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review. Journal of Hazardous Materials 169(1-3):1-15.

International Agency for Research on Cancer (IARC) (1987). IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans, suppl. 7: overall evaluations of carcinogenicity-an updating of IARC monographs volumes 1 to 42. International Agency for Research on Cancer, Lyon, France.

International Agency for Research on Cancer (IARC) (2000). IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans, vol. 77: some industrial chemicals. International Agency for Research on Cancer, Lyon, France.

Lu ST, Kaplan IR (2008). Characterization of motor lubricating oils and their oil water partition. Environmental Forensics 9(4):295-309.

Marchant R, Banat IM (2012). Microbial biosurfactants: challenges and opportunities for future exploitation. Trends in Biotechnology 30(11):558-565.

Marchut-Mikolajczyk O, Drozdzynski P, Pietrzyk D, Antczak T (2018). Biosurfactant production and hydrocarbon degradation activity of endophytic bacteria isolated from *Chelidonium majus* L. Microbial Cell Factories 17:171. https://doi.org/10.1186/ s12934-018-1017-5

Millioli VS, Servulo ELC, Sobral LGS, De Carvalho DD (2009). Bioremediation of crude oil-bearing soil: evaluating the effect of rhamnolipids addition to soil toxicity and to crude oil biodegradation efficiency. Global Nest Journal 11(2):181-188.

Mnif I, Mnif S, Sahnoun R, Maktouf S, Ayedi Y, Chaabouni SE, Ghribi D (2015). Biodegradation of diesel oil by a novel microbial consortium: comparison between co-inoculation with biosurfactant-producing strain and exogenously added biosurfactants. Environmental Science and Pollution Research 22(19):14852-14861.

Parthipan P, Preetham E, Machuca LL, Rahman PKSM, Murugan K, Rajasekar A (2017). Biosurfactant and degradative enzymes mediated crude oil degradation by bacterium *Bacillus subtilis* A1. Frontiers in Microbiology 8:193. doi:10.3389/fmicb.2017.00193.

Speight JG, Arjoon KK (2012). Bioremediation of petroleum and petroleum products. Scrivener Publishing, Beverly, Massachusetts, USA.

Whang LM, Liu PWG, Ma CC, Cheng SS (2008). Application of biosurfactants, rhamnolipid, and surfactin, for enhanced biodegradation of diesel-contaminated water and soil. Journal of

Hazardous Materials 151(1):155-163.
Yakimov MM, Denaro R, Genovese M, Cappello S, D'Auria G, Chernikova TN, Timmis KN, Golyshi PN (2005). Natural microbial diversity in superficial sediments of Milazzo Harbor (Sicily) and community successions during microcosm enrichment with various hydrocarbons. Environmental Microbiology 7(9):1426-1441.