ISSN 1226-9999 (print)
ISSN 2287-7851 (online)

Korean J. Environ. Biol. 36(2) : 199~205 (2018) https://doi.org/10.11626/KJEB.2018.36.2.199

〈Original article〉

Investigating Biochemical Properties of *Bacillus aryabhattai* DA2 from Diesel-Contaminated Soil

Sang-Jun Kim, Arjun Adhikari[1], Ko-Eun Lee[1] and Gil-Jae Joo[2,*]

Department of Natural Sciences, Naval Academy, Changwon 51698, Republic of Korea
[1]*School of Applied Biosciences, Kyungpook National University, Daegu 41566, Republic of Korea*
[2]*Institute of Agricultural Science and Technology, Kyungpook National University, Daegu 41566, Republic of Korea*

Abstract - Petroleum energy is the major source of the world energy market, and its massive usage, and the corresponding extreme environmental pollution, imposes a serious threat on the ecological cycles. By screening oil-contaminated soil, we isolated, identified, and characterized a novel strain that represents a considerable diesel-degrading potentiality; the *Bacillus aryabhattai* DA2 strain is registered in the NCBI with the accession number MG571630, and it possesses an efficient tributyrin-degrading capacity. The optimal condition for diesel degradation by DA2 strain was observed at pH between 7-8 and at the temperature of 30°C. The strain is resistant to salt as well as the antibiotics like ampicillin and streptomycin. These results indicate *B. aryabhattai* is one of the potential candidates for the remediation of the diesel-contaminated sites.

Keywords : *Bacillus aryabhattai*, biochemical, biodegradation, diesel, pollution

INTRODUCTION

Petroleum energy is a top energy resource and comprises a major portion of the global economy (Cerqueira *et al*. 2011). It also has adverse effects on the environment arousing a big challenge to mitigate environmental problems from its products (Deng *et al*. 2014). It was reported that almost 600,000 metric tons of natural crude oil are released through seepage alone (Kvenvolden and Cooper 2003). Cytoalkanes, aromatic hydrocarbons, and n-alkanes are the most common agents of petroleum hydrocarbon responsible for environmental pollution (Deng *et al*. 2014).

Petroleum is exposed to the environment during transportation and industrial leakage (Wei *et al*. 2005). A microbial mediated biodegradation process is one of the globally accepted and biologically safe measures to replenish the environment after petroleum pollution (Cappello *et al*. 2007; Kubota *et al*. 2008). These processes include microbes that can degrade the long-chain alkanes (Wentzel *et al*. 2007). It has been reported that several microorganisms like *Stenotrophomonas maltophilia*, *Stenotrophomonas acidaminiphila*, *Bacillus cibi*, *Bacillus pumilus*, *Bacillus megaterium*, and *Pseudomonas aeruginosa* are involved in biodiesel degradation (Cerqueira *et al*. 2011; Meyer *et al*. 2012).

Biodegradation is the most economically viable technology to remove petroleum hydrocarbons compared to other techniques (Das and Chandran 2011). The rate of biodegradation depends on numerous factors including oil concentration and composition. The biodegradation rate can be ideally achieved if the appropriate conditions are maintained in the contaminated sites (Leahy and Colwell 1990). There are several species involved in diesel degradation like *Arthrobacter sulphurous*, *Brevibacterium* sp., *Pseudomonas* sp., and *Acidovorax delafieldii* (Samanta *et al*. 1999), *Beijerinckia* Bwt, *Alcaligenes faecalis*, *Pseudomonas* SPM64 (Kiyo-

* Corresponding author: Gil-Jae Joo, Tel. 053-950-6854, Fax. 053-953-6972, E-mail. gjjoo@knu.ac.kr

hara *et al*. 1982), and *Mycobacterium* sp. strain BB1 (Boldrin *et al*. 1993).

Microrganisms were found to get involved in the production of biosurfactants that enhance oil recovery from the contaminated sites (Geetha *et al*. 2018). They have promising features such as they enhance higher biodegradability and lower toxicity. Moreover, they can sustain in extreme pH level, temperature and salinity (Mukherjee *et al*. 2006). Surfactants produced by microorganism could effectively degrade polycyclic aromatic hydrocarbons (PAHs) through transformation of PAHs to an aqueous phase from a solid phase. The process involves an increase in the surface hydrophobicity of microbial cells to uptake PAHs through solubility of the micelle (Li and Zhu 2012). Numerous biosurfactant-producing strains have been identified to date. Still, use in practice is confined to the chemically synthesized surfactants (Mukherjee *et al*. 2006). Also the bacterial lipases are in great demand because of potential industrial applications (Sirisha *et al*. 2010). Lipases are triacylglycerol hydrolases that catalyze the hydrolysis of triacylglycerol to glycerol and fatty acids (Sharma *et al*. 2001). The most suitable sources for lipase production are microbes including bacteria, fungi, and yeast (Mahima *et al*. 2016).

Since environmental pollution from petroleum products is an escalating problem, the potential of hydrocarbon-degrading microorganisms for bioremediation of contaminated water and soil is promising and is gaining momentum (Dua *et al*. 2002). Identification of a microorganism to facilitate the process might be involved in a future solution and has the potential for large-scale application in biodegradation processes. With this in mind, we describe one efficient candidate that has a very high potential for diesel degradation.

MATERIALS AND METHODS

1. Isolation of diesel degrading bacteria in the crude oil

To isolate the bacterial strain, 10 g of each contaminated soil sample was diluted in 100 mL of distilled water for 24 hours at 30°C and 150 rpm and then was cultured in an Minimal Salt Medium (Table 1), containing 1% diesel at 30°C for a week. Then, 100 μL of the media was plated on a solid medium (MSM broth, diesel 1%, agar 1.5%) and cultured until the colony formed. After that, the media subculture was mixed with sterilized glycerol (20%) and stored at −80°C (Choi *et al*. 2010).

2. Lipolytic activity assay

Lipid degradation activity was determined by inoculating a strain in a marine (Difco, USA) medium supplemented with agar and 1% tributyrate (Tributyrin, TBN; C4; Sigma, USA) at 28°C for five days. The strain was selected on the basis of its expression to form a clear zone (Kwon *et al*. 2015). Protein degradation activity was determined by a sterilized medium of skim milk (Difco, USA, 0.5% agar).

3. Antibiotic resistivity performance test

The antibiotic-resistant ability of the bacteria was tested by investigating the microbial growth on these various antibiotics: rifampin, ampicillin, penicillin, kanamycin, and streptomycin. The culture media were inoculated in an antibiotic-susceptible disk placed in an MSM media and incubated at 28°C. A transparency formation was determined to confirm the resistivity to antibiotics.

Table 1. Compositions of the minimal salt medium (MSM) for the bacteria culture.

Components	Concentration (g L^{-1})	Components of trace element solution	Concentration (g L^{-1})
Na_2HPO_4	0.86	$ZnSO_4 \cdot 7H_2O$	2.32
$NaNO_3$	0.85	$MnSO_4 \cdot 7H_2O$	1.78
KH_2PO_4	0.56	$CuSO_4 \cdot 5H_2O$	1.0
$MgSO_4 \cdot 7H_2O$	0.37	KI	0.66
K_2SO_4	0.17	H_3BO_3	0.56
$CaCl_2 \cdot H_2O$	0.007	$Na_2MoO_4 \cdot 2H_2O$	0.39
Fe (III) EDTA	0.004	EDTA	1.0
Trace element solution	0.25 mL	$FeSO_4 \cdot 7H_2O$	0.4
		$NiCl \cdot 6H_2O$	0.004

4. Salt resistivity performance test

To test the salt resistivity performance of the microbes the MSM media was supplemented with 5% and 7% NaCl. The bacterial cultures were inoculated and similarly incubated as in the above section. The microbial growth was determined to confirm the salt resistant ability of the strain.

5. Selection, identification and phylogenetic tree construction

The strain that had the highest potential for lipase activity, and antibiotic and salt resistant ability was selected and named DA2 for identification and further investigation. To amplify the 16S rRNA region of the strain, a 518f primer (5′-CCA GCA GCC GCG GTA ATA C -3′) and an 805r primer (5′- GAC TAC CAG GGT ATC TAA TC -3′) were used as templates for PCR. Enlarged products were obtained to determine the base sequence. The determined nucleotide sequence was identified by homology searching through BLAST run by NCBI MEGA 6 version and a phylogenetic tree was constructed using maximum parsimony 1000 bootstrap.

6. Determination of optimal conditions for diesel degradation

The bacteria was cultured for 12 hours in MSM media and then centrifuged to about 7,000 rpm. The obtained cells were washed twice with 25 mM phosphate buffer. Then the cells were diluted to 1 : 1000 into freshly prepared MSM media mediated with diesel fuel 0.1% that was used as the sole carbon source. The bacterial culture was grown at 35°C with agitation for 72 hours. The samples were plated for determining the colony formation and the number of units (Gran-Scheuch *et al*. 2017).

7. Residual diesel component analysis

The 2% of pre-culture broth was inoculated in the crude liquid medium then supplemented with 1% diesel and incubated for 4 days at 30°C and 180 rpm. The methodology described by Dibble and Bartha (1976) was followed for the sample treatment. The crude liquid was added to the separating funnel then mixed well with n-hexane to transfer it into the n-hexane layer. This process was repeated three times and the oil in the culture broth was completely transferred to the n-hexane layer. The filtrate was recovered and filtered through a chemical analysis filter paper (Watman No.13) filled with 20 g of anhydrous sodium sulfate. The resulted filtrate was used for the analysis.

8. Enzyme activity assay

Determination of Catechol 1,2-dioxygenase activities was performed by the method described by Liu *et al*. (2002). The unit of catechol dioxygenase activity is the amount of protein required for the oxidation of 1 μmol of catechol per minute. The unit is expressed as U/L (U-one enzymatic unit, L-volume of reaction mixture).

9. Biochemical assay

The analysis of various substrate production with different reaction enzymes was tested for the following: gram stain, catalase, oxidase, potassium nitrate, tryptophan, glucose, arginine, urea, esculin, gelatin, p-Nitrophenyl-β-D-galactopyranoside, glucose, arabinose, mannose, mannitol, N-acetyl-glucosamine, maltose, gluconate, Caproate, adipate, malate, citrate, and phenylacetate. The catalase and oxidase test and Gram staining of the strain were performed by the method described by Collins *et al*. (1970). And the remaining biochemical tests were performed using API 20NE test strip (BioMerieux Co., France).

10. Statistical analysis

The experiment was independently repeated three times and was comprised of three replications per treatment. The data were statistically analyzed with SAS 9.4 software (SAS Institute, Cary, NC, USA). The data from these experiments were pooled together and subjected to Duncan's multiple range test: $p \leq 0.05$.

RESULTS AND DISCUSSION

1. Isolation, selection, and identification of oil-degrading microorganisms

Through screening of oil contaminated soil from petrol stations in Jinhae, Gyeongnam we obtained three strains in-

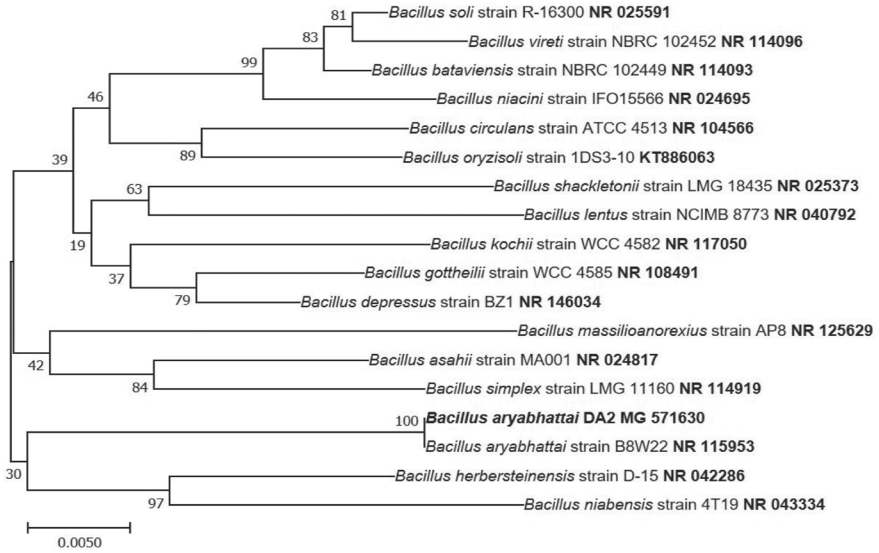

Fig. 1. Phylogenetic tree of the bacterial isolate *Bacillus aryabhattai* DA2 (MG571630) based on the 16S rRNA gene sequence of the DA2 and the related bacteria.

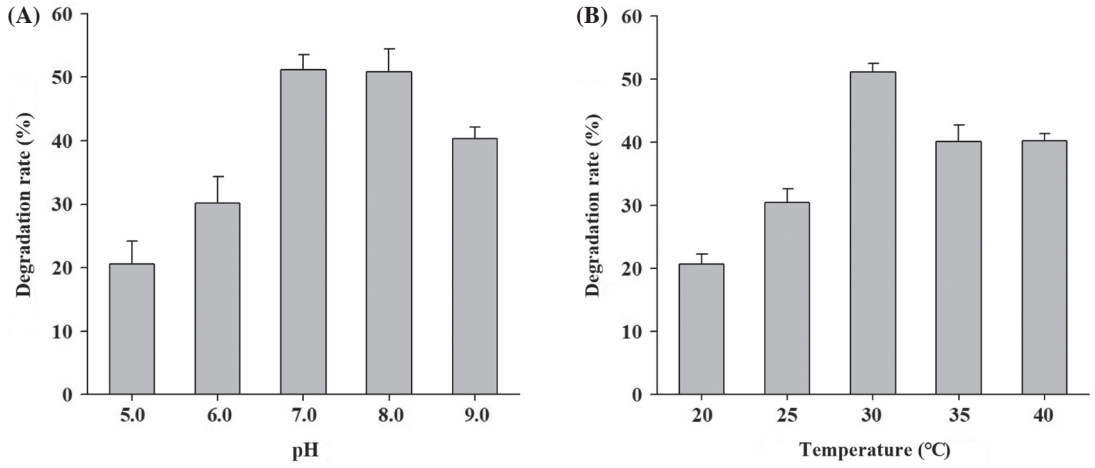

Fig. 2. Influence of (A) pH and (B) temperature on the degradation of the diesel that was used as the sole carbon source of the *Bacillus aryabhattai* DA2. The bars represent means ± SD ($n = 3$).

cluding: *Bacillus toyonensis* ONT8, *Bacillus subtilis* ONM 10, and *Bacillus aryabhattai* ONM33. And we selected the highest potential strain that was involved in petroleum degradation. The obtained sequence of isolated strain analyzed through 16S rRNA gene sequencing was used to construct the phylogenetic tree. The tree was constructed through the nucleotide sequence obtained from blast search run by NCBI (Mega version 6). The strain represents 100% homology to *Bacillus aryabhattai*. The phylogenetic tree was constructed by aligning a similar sequence using the Clustal W. MEGA ver 6.0. A 1000 Bootstrap replication was used for the robust statistical support in each node of the phylogenetic tree (Fig. 1).

2. Determination of optimal stage for degradation

In our study, the diesel degradation rate of the DA2 strain was significantly higher at the pH levels ranging from 7−8 compared to the pH levels above or below this range (Fig. 2). Similarly, the maximum degradation rate was achieved at the temperature 30°C as compared to temperatures above or below this level. Previously, it was reported that the pH

levels ranging from 6−9 is optimal to gain maximum degradation of hydrocarbon (Das and Chandran 2011). Wongsa *et al*. (2004) reported that microorganisms like *Pseudomonas aeruginosa* and *Serratia marcescens* degrade diesel up to 90−95% within 2−3 weeks. The solubility of the hydrocarbons was influenced by the temperature (Foght *et al*. 1996). It was also reported that the biodegradation rate decreases with decreases in temperature. The optimal temperature for biodegradation in a soil environment ranges in between 30−40°C (Bartha and Bossert 1984; Das and Chandran 2011).

3. Antibiotic and salinity tests

The antibiotic and salinity tests performed on various isolated strains are represented in Table 1. Among them the DA2 strain is susceptible to rifampin, penicillin, kanamycin, and resistant to ampicillin and streptomycin. The ability to resist the salt (5% and 7%) is higher in the DA2 strain compared to the DA8 and DA13 strains.

4. Determination of lypolytic activity

Tributyrin was examined to determine the degradability of fats. In our study, the DA2 strain possesses both the ability to degrade the tributyrin and proteins (Table 2). It was reported previously that microorganisms having lipid-degrading ability could be used as an environmentally-friendly agent for purification as an alternative way of oil removing chemical agents during oil accidents (Seo *et al*. 2006).

5. Biochemical characteristics

The strain was able to produce catalase, potassium nitrate, glucose, esculin, arabinose, mannose, N-acetyl-glucosamine, maltose, adipate, malate, citrate, and phenylacetate. Whereas, the strain showed no growth/production of oxidase, tryptophan, arginine, urea, gelatin, p-Nitrophenyl-β-D-galactopyranoside, Mannitol, gluconate, and caproate. All the metabolites are regulated by different enzymatic actions or reactions (Table 3).

Table 2. Antibiotic resistance and tributyrin tests of the diesel-degrading bacteria

Degrading bacteria	Skim milk	Tributyrin	Antibiotic resistance				
			R	Am	P	K	S
DA2	○	○	s	r	s	s	r
DA8	−	−	s	s	s	r	r
DA13	○	−	r	r	r	s	s

R: rifampin; Am: ampicillin; P: penicillin; K: kanamycin; S: streptomycin
r: resistance; s: sensitivity

Table 3. Biochemical characteristics of the isolated DA2.

Substrates	Reaction/Enzyme	DA2
Gram stain		Negative
Catalase		+
Oxidase		−
Potassium nitrate	Reduction of nitrate to nitrite	+
Tryptophan	Indole production	−
Glucose	Acidification	+
Arginine	Arginine dihydrolase	−
Urea	Urease	−
Esculin	Hydrolysis (β-glucosidase)	+
Gelatin	Hydrolysis (protease)	−
p-Nitrophenyl-β-D-galactopyranoside	β-galactosidase	−
Glucose	Glucose assimilation	+
Arabinose	Arabinose assimilation	+
Mannose	Mannose assimilation	+
Mannitol	Mannitol assimilation	−
N-acetyl-glucosamine	N-acetyl-glucosamine assimilation	+
Maltose	Maltose assimilation	+
Gluconate	Gluconate assimilation	−
Caproate	Caproate assimilation	−
Adipate	Adipate assimilation	+
Malate	Malate assimilation	+
Citrate	Citrate assimilation	+
Phenylacetate	Phenylacetate assimilation	+

6. Catechol 1,2-dioxygenase activities

An estimated one-third of the bacteria involved in the hydrocarbon degradation possess a catabolic pathway for aliphatic and aromatic hydrocarbons (Margesin *et al*. 2002). The catabolic activities of microorganisms play an important role in the degradation of pollutants (Cheung and Kinkle 2001). Aitken *et al*. (1998) reported that the most *Bacillus*, *Agrobacterium Burkholderia*, *Sphingomonas*, and *Pseudomonas* could oxidize compounds like phenanthrene. In our study, we found that the Catechol 1,2-dioxygenase activities of the DA2 strain multiplied rapidly from the second day of incubation and reached a maximum in about 10 days and started declining from the 10^{th} day (Fig. 3). The possible synthesis of catechol 1,2-dioxygenase activities involved in petroleum degradation is through the increase in cell surface hydrophobicity of the microorganisms that enhance the electron transport system and catechol 1,2-dioxygenase

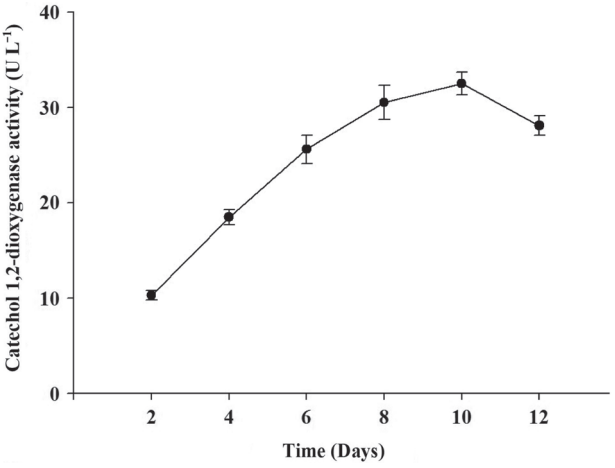

Fig. 3. The potentiality of the *Bacillus aryabhattai* DA2 regarding the performance of the activities of the catechol 1,2-dioxygenase in different time periods.

activities, which finally degrade the petroleum compounds (Li and Zhu 2012). Moreover the enzymatic activities are influenced by several factors like pH and temperature (Nadaf and Ghosh 2011; Guzik *et al*. 2013). Thus, influence of favorable pH and temperature condition might have enhanced the catechol 1,2-dioxygenase activity of *Bacillus aryabhattai* DA2.

CONCLUSION

Massive industrialization and other anthropogenic activities led to environmental contamination and are increasing almost daily. The approach to tackle pollutants responsible for natural threats from different scientist and researchers is still an unsolved paradox. The global demand for the remediation of environmental pollution calls for economically viable, environmentally sound, and socially acceptable approaches. Our research identified the microbial strain involved in biodegradation that has the most potential to be applied on a large scale for the remediation of pollutants with an eco-friendly approach.

REFERENCES

Aitken MD, WT Stringfellow, RD Nagel, C Kazunga and SH Chen. 1998. Characteristics of phenanthrene-degrading bacteria isolated from soils contaminated with polycyclic aromatic hydrocarbons. Can. J. Microbiol. 44:743–752.

Alvarez PJ and TM Vogel. 1991. Substrate interactions of benzene, toluene, and para-xylene during microbial degradation by pure cultures and mixed culture aquifer slurries. Appl. Environ. Microbiol. 57:2981–2985.

Bartha R and I Bossert. 1984. The treatment and disposal of petroleum wastes.

Boldrin B, A Tiehm and C Fritzsche. 1993. Degradation of phenanthrene, fluorene, fluoranthene, and pyrene by a *Mycobacterium* sp. Appl. Environ. Microbiol. 59:1927–1930.

Cappello S, G Caruso, D Zampino, LS Monticelli, G Maimone, R Denaro and L Giuliano. 2007. Microbial community dynamics during assays of harbour oil spill bioremediation: a microscale simulation study. J. Appl. Microbiol. 102:184–194.

Cerqueira VS, EB Hollenbach, F Maboni, MH Vainstein, FA Camargo, RP Maria do Carmo and FM Bento. 2011. Biodegradation potential of oily sludge by pure and mixed bacterial cultures. Bioresour. Technol. 102:11003–11010.

Cheung PY and BK Kinkle. 2001. *Mycobacterium* diversity and pyrene mineralization in petroleum-contaminated soils. Appl. Environ. Microbiol. 67:2222–2229.

Choi HC, YA Cho, SI Choi and TJ Lee. 2010. A study on microbial community and microbial degradation of diesel. J. Korean Soc. Environ. Eng. 32:509–516.

Collins CH and PM Lyne. 1970. Microbiological methods (3rd ed.). University Park press, Butterworths Baltimore, London.

Das N and P Chandran. 2011. Microbial degradation of petroleum hydrocarbon contaminants: an overview. Biotechnol. Res. Int. 2011:ID941810.

Deng MC, J Li, FR Liang, M Yi, XM Xu, JP Yuan, J Peng, CF Wu and JH Wang. 2014. Isolation and characterization of a novel hydrocarbon-degrading bacterium *Achromobacter* sp. HZ01 from the crude oil-contaminated seawater at the Daya Bay, southern China. Mar. Pollut. Bull. 83:79–86.

Dibble JT and R Bartha. 1976. Effect of iron on the biodegradation of petroleum in seawater. Appl. Environ. Microbiol. 31:544–550.

Dua M, A Singh, N Sethunathan and A Johri. 2002. Biotechnology and bioremediation: successes and limitations. Appl. Microbiol. Biotechnol. 59:143–152.

Foght JM, DW Westlake, WM Johnson and HF Ridgway. 1996. Environmental gasoline-utilizing isolates and clinical isolates of *Pseudomonas aeruginosa* are taxonomically indistinguishable by chemotaxonomic and molecular techniques. Microbiology 142:2333–2340.

Geetha SJ, IM Banat and SJ Joshi. 2018. Biosurfactants: Pro-

duction and potential applications in microbial enhanced oil recovery (MEOR). Biocatal. Agric. Biotechnol. 14:23–32.

Gran-Scheuch A, E Fuentes, DM Bravo, JC Jiménez and JM Pérez-Donoso. 2017. Isolation and characterization of phenanthrene degrading bacteria from diesel fuel-contaminated Antarctic soils. Front. Microbiol. 8:1634.

Guzik U, K Hupert-Kocurek, M Sitnik and D Wojcieszyńska. 2013. High activity catechol 1,2-dioxygenase from Stenotrophomonas maltophilia strain KB2 as a useful tool in cis, cis-muconic acid production. Antonie Leeuwenhoek 103: 1297–1307.

Kiyohara H, K Nagao and K Yana. 1982. Rapid screen for bacteria degrading water-insoluble, solid hydrocarbons on agar plates. Appl. Environ. Microbiol. 43:454–457.

Kubota K, D Koma, Y Matsumiya, SY Chung and M Kubo. 2008. Phylogenetic analysis of long-chain hydrocarbon-degrading bacteria and evaluation of their hydrocarbon-degradation by the 2, 6-DCPIP assay. Biodegradation 19:749–757.

Kvenvolden K and C Cooper. 2003. Natural seepage of crude oil into the marine environment. Geo-Mar. Lett. 23:140–146.

Kwon TH, JH Woo, NH Park and JS Kim. 2015. Characterization of PAH (Polycyclic Aromatic Hydrocarbon)-degrading bacteria isolated from commercial gasoline. Korean J. Environ. Agric. 34:244–251.

Leahy JG and RR Colwell. 1990. Microbial degradation of hydrocarbons in the environment. Microbiol. Rev. 54:305–315.

Li F and L Zhu. 2012. Effect of surfactant-induced cell surface modifications on electron transport system and catechol 1,2-dioxygenase activities and phenanthrene biodegradation by *Citrobacter* sp. SA01. Bioresour. Technol. 123:42–48.

Liu Z, H Yang, Z Huang, P Zhou and SJ Liu. 2002. Degradation of aniline by newly isolated, extremely aniline-tolerant *Delftia* sp. AN3. Appl. Microbiol. Biotechnol. 58:679–682.

Mahima G, H Krishnan and GP Pandey. 2016. Screening, identification, characterization and production of bacterial lipase from oil spilled soil. Int. J. Curr. Microbiol. App. Sci. 5:745–763.

Margesin R, G Feller, M Hämmerle, U Stegner and F Schinner. 2002. A colorimetric method for the determination of lipase activity in soil. Biotechnol. Lett. 24:27–33.

Meyer DD, NA Santestevan, F Bücker, SP Salamoni, R Andreazza, FA De Oliveira Camargo and FM Bento. 2012. Capability of a selected bacterial consortium for degrading diesel/biodiesel blends (B20): enzyme and biosurfactant production. J. Environ. Sci. Health Part A 47:1776–1784.

Mukherjee S, P Das and R Sen. 2006. Towards commercial production of microbial surfactants. Trends Biotechnol. 24:509–515.

Nadaf NH and JS Ghosh. 2011. Purification and characterization of catechol 1, 2-dioxygenase from *Rhodococcus* sp. NCIM 2891. Res. J. Environ. Earth Sci. 3:608–613.

Samanta SK, AK Chakraborti and RK Jain. 1999. Degradation of phenanthrene by different bacteria: evidence for novel transformation sequences involving the formation of 1-naphthol. Appl. Microbiol. Biotechnol. 53:98–107.

Sharma R, Y Chisti and UC Banerjee. 2001. Production, purification, characterization and application of lipases. Biotechnol. Adv. 19:627–662.

Sirisha E, N Rajasekar and LM Narasu. 2010. Isolation and optimization of lipase producing bacteria from oil contaminated soils. Adv. Biol. Res. 45:249–252.

Seo JS, YS Keum, Y Hu, SE Lee and QX Li. 2006. Phenanthrene degradation in *Arthrobacter* sp. P1-1: initial 1, 2-, 3, 4- and 9, 10-dioxygenation, and meta- and ortho-cleavages of naphthalene-1, 2-diol after its formation from naphthalene-1, 2-dicarboxylic acid and hydroxyl naphthoic acids. Chemosphere 65:2388–2394.

Wei Q, R Mather and A Fotheringham. 2005. Oil removal from used sorbents using a biosurfactant. Bioresour. Technol. 96:331–334.

Wentzel A, TE Ellingsen, HK Kotlar, SB Zotchev and M Throne-Holst. 2007. Bacterial metabolism of long-chain n-alkanes. Appl. Microbiol. Biotechnol. 76:1209–1221.

Wongsa P, M Tanaka, A Ueno, M Hasanuzzaman, I Yumoto and H Okuyama. 2004. Isolation and characterization of novel strains of *Pseudomonas aeruginosa* and *Serratia marcescens* possessing high efficiency to degrade gasoline, kerosene, diesel oil, and lubricating oil. Curr. Microbiol. 49:415–422.

Received: 8 December 2017
Revised: 8 June 2018
Revision accepted: 11 June 2018