Isolation and Characterization of a Petroleum-Degrading *Pseudoalteromonas Haloplanktis* Strain from the Digestive Tract of *Perinereis Aibuhitensis* (Polychaete)

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Abstract: The application of bioremediation approaches employing hydrocarbon-utilizing microorganisms to remove petroleum hydrocarbons from oil spills is an area of research that has gained extensive attention and has been widely investigated. In the present study, attempts have been made to isolate and characterize hydrocarbon-utilizing microorganisms immobilized in the digestive tract of *Perinereis aibuhitensis*. Isolate SC11-3, a potent petroleum-degrading organism, from *Perinereis aibuhitensis* gut samples was identified as *Pseudoalteromonas* sp. A detailed morphological, biochemical, and 16S rDNA sequence analysis revealed that it was closely related to *Pseudoalteromonas haloplanktis*. The isolate SC11-3 was capable of consuming about 40% diesel within 15 days from the medium containing 1 ml L^{-1} of oil. Furthermore, it was observed that the degrading efficiency of the isolate SC11-3 was significantly enhanced up to approximately 90% when the medium was supplemented with 4 g L^{-1} of glucose, indicating the possible occurrence of co-metabolism during the process of petroleum degradation by the bacterium. Our study reported an isolate of petroleum-degrading bacterium and its potential co-metabolism mechanism in oil degradation processes, which will provide new insight into in situ bioremediation of multi-biological systems.

1 INTRODUCTION

Marine contamination has become a major concern as a result of increasing demand for imported petroleum fuels and growing exploitation of marine petroleum oil sources. Oil spill from transport pipelines, storage tanks, and petroleum exploitation could seriously pollute the marine environment and disturb the surrounding ecosystem, mainly the intertidal zone of the shoreline. Petroleum hydrocarbon components are known to belong to the family of neurotoxic and carcinogenic organic contaminants (Nilanjana and Preethy 2011). Bioremediation is considered as eco-friendly and economic method to control petroleum pollution owing to its advantages such as cost-effectiveness and complete mineralization (Balba et al. 1998; Vergeynst et al. 2018).

Recent studies have paid much attention to the applications of bioremediation approaches employing hydrocarbon-utilizing microorganisms to remove petroleum hydrocarbon pollutants. Microorganisms such as bacteria, fungi, yeasts, and microalgae have the ability to mineralize petroleum hydrocarbons (Atlas 1981; Leahy and Colwell 1990; Ortega-González et al. 2015; Santos and Maranho 2018). However, the dilution of seeded microorganisms or fertilizers is considered as one of the major limitation for the application of bioremediation processes (Radwan et al. 2002; Panchal et al. 2018). As a result, there has been an increasing interest in the use of marine sedimentary invertebrates associated with petroleum-utilizing bacteria in bioremediation as in situ multi-biological

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approach for cleaning polluted marine environments. There have been a few reports on organisms that can degrade petroleum hydrocarbons or other high molecular weight (HMW) polycyclic aromatic hydrocarbons (PAHs), colonized in soil or other bio-carriers, such as marine white-rot fungi and autochthonous microflora (Radwan et al. 2002; Wen et al. 2011).

During the past few decades the incidence and threat of anthropogenic origins of petroleum pollution has led to extensive research in isolation and characterization of oil-degrading microorganisms, particularly for the marine environment. Most bacterial petroleum hydrocarbon degraders have been isolated from heavily contaminated coastal areas (Ridgway et al. 1990; Mikesell et al. 1993, 1994; Watanabe et al. 1998; Itagaki and Ishida, 1999; Kasai et al., 2001; Tazaki, 2003; Chaerun et al. 2004; Vergeynst et al. 2018;). However, few studies have concentrated on hydrocarbon-utilizing microorganisms immobilized in the digestive tract of marine sedimentary invertebrates, which exhibit high resistance to pollution. Invertebrates participate actively in the interactions that develop in sediment among physical, chemical and biological processes, which play significant roles in the delivery of ecosystem services (Lavellea et al. 2006). Plants, invertebrates and microorganisms have coevolved over several hundred million years within soils. Invertebrates are generally considered as key actors in the buffering systems, which creates biogenic structures that may act as incubators of microbial activities or microsites for carbon and nutrient sequestration (Lavellea et al. 2006). For example, intestinal mucus plays a significant role in the selection and stimulation of microbial activities in the earthworm guts (Barois and Lavelle 1986) and the effects of earthworm cutaneous mucus on microbial selection have also been demonstrated (Lavelle et al. 2005). Polychaetes from the intertidal zone are known to accumulate significant amounts of organic matter in addition to biotransformation and elimination processes, which make Polychaetes as candidate promoters and indicators of oil-degrading mutualistic micro-organisms.

Polychaetes present a wide geographical distribution. Owing to their characteristics such as short-distance migration and steady-state body burden, polychaetes are known to accumulate significant amounts of organic matter from the environment and possess the ability to carry out biotransformation and elimination processes (Chen et al. 2012; Jørgensen et al. 2008). It is known that the microbiota of the digestive tract has a crucial impact on the host, and the interactions between invertebrates and microorganisms are essential for the bioremediation of marine sedimentary environment because they affect organic matter degradation and nutrient cycling (Byzov et al. 2007; Knapp et al. 2009). Thus, the purpose of this study was to isolate and identify potential petroleum-degrading bacteria from the digestive tract of *P. aibuhitensi*s, and provide useful insight into in situ bioremediation of multi-biological systems. In addition, the capability of *Pseudoalteromonas haloplanktis* to degrade diesel along with glucose as a supplemented co-substrate of carbon source for diesel degradation was also investigated. The results of our study could be helpful in exploring the possibility of cleaning polluted marine environments in a more efficient way.

2 MATERIALS AND METHODS

2.1 Sample Collection and Isolation of Microorganisms

Hydrocarbon-utilizing bacteria were isolated from gut samples of P. aibuhitensis collected from the shoreline of Panjin (Liaoning Province, China). Live worms weighing approximately 5 g were transported to the laboratory, and after being starved for 24 h, the gut samples were removed using sterile forceps and scissors on a super-clean bench. For the isolation of hydrocarbon-utilizing bacteria, the diluted homogenate of the gut samples was serially diluted in sterile distilled water and daubed on solid mineral medium (sterilized by autoclaving at 121℃, 15 psi for 15 min) supplemented with 1% (v/v) sterile diesel as the sole carbon source. Subsequently, the plates were incubated at 25°C for 5 days and screened for hydrocarbon-utilizing bacterial colonies.

2.2 Identification and Characterization of the Bacterial Isolates

The selected isolates were grown on 2216E agar medium (peptone 5g, yeast extract 1g, powdered agar 15g, Ferric phosphate 0.01g, seawater 1L, pH7.6-7.8 and sterilized by autoclaving at 121℃, 15 psi for 15 min). The shape and colors of the colonies

were screened out by observing bacterial form properties of colony. In addition, the isolates were also biochemically analyzed by conducting oxidase, catalase, urease, V-P (Voges-Proskauer test), MR-VP (methyl red test), nitrate reduction, oxidative fermentation (OF), arginine dehydrolase, gelatin hydrolysis, motility, glucose and citrate utilization, TCBS (thiosulphate citrate bile salts) growth, and O/129 drug susceptibility tests (Table 1). All the identification tests were carried out according to Bergey's Manual of Systematic Bacteriology (Williams and Wilkins 1986) and A Practical Identification Manual of Bacteria from Fish and Other Aquatic Animals (Nicky 2004).

2.3 Determination of Optimal Growth Conditions

The optimal growth conditions with reference to pH, temperature, and saline concentration were determined. The strains were grown in 5 ml of medium at varying pH values (5, 6, 7, 8, 9, and 10), at different temperatures (5, 10, 15, 20, 25, 30, and 40°C), and with various NaCl concentrations (0%, 1%, 2%, 3%, 4%, and 5%), respectively. All treatments were carried out in triplicate for 24 h with shaking at 150 rpm. The optical densities of the growing biomass under all the above-mentioned conditions were assessed at 600 nm using an UV-Vis spectrophotometer to determine the optimum growth.

2.4 16s Rdna Sequencing, Alignment, and Phylogeny

The isolates were purified using streaking method before being subjected to sequencing (TaKaRa Biotechnology (Dalian) Co., Ltd.). The full length of the 16S rRNA genes (1450 bp) of the isolates was amplified and sequenced using TaKaRa 16S rRNA Bacterial Identification PCR Kit. The sequences were analyzed for homology to other known sequences matched with previously published bacterial 16S rDNA sequences using the BLAST program (Basic Local Alignment Search Tool). Based on the scoring index, the most similar sequences were aligned with the sequences of other representative bacterial 16S rDNA regions (Woese and Fox 1977), and a phylogenetic tree was constructed using the neighbor-joining method with Bootstrap of 1000.

2.5 Estimation of Bacterial Petroleum-Degrading Efficiency

The bio-utilization of diesel was examined by using fresh bacterial suspension (approximately 2×10^9 cfu/ml; 2% (v/v)) grown in 250-ml conical flasks containing 100 ml of MMC medium (minimal medium 1000ml, powdered agar 15g, Tween 80 1ml, pH7.2 and sterilized by autoclaving at 121℃, 15 psi for 15 min) supplemented with 0.1 ml of sterile diesel. The flasks were incubated on a rotary shaker at 150 rpm and 25°C for 3, 5, 7, 10, and 15 days, respectively and MMC medium without inoculum was experimented as control group. All treatments were carried out in triplicate, and the residual oil of the samples was extracted at selected time intervals by using petroleum ether (transmittance: >90%; boiling range: 60–90°C). The residual oil present in the solution was determined by UV spectrophotometry at 221 nm (standard curve was established by employing sterile diesel and petroleum ether; R2=0.9995). The degradation rates of the samples were estimated by using the MMC medium without bacterial inoculum as control.

2.6 Effect of Glucose on Bacterial Degrading Efficiency

The potentiality of microorganisms associated with different concentrations of glucose as the supplemented source of carbon for petroleum degradation in sea water was determined quantitatively. For this experiment, two different inoculum concentrations were employed: about 0.5% (v/v) or 2% (v/v) (approximately 0.5 ml and 2.0 ml, respectively) of fresh bacterial suspension (approximately 2×10^9 cfu ml-1) was added to each flask containing 100 ml of the MMC medium with 0.1% (v/v) sterile diesel. Then, the flasks were incubated on a rotary shaker at 150 rpm and 25°C for 3 days. Three replicates were prepared for each inoculum and glucose concentration (glucose concentrations of 0.5, 1, 2, 4, 6, 8, 10, 16, 24, 32, and 40 g L-1 for 0.5-ml inoculum, respectively; glucose concentrations of 0.5, 1, 2, 4, 6, 8, 10, 16, 32, 48, 64, 80, and 96 g L-1 for 2.0-ml inoculum, respectively). The number of oil-utilizing microorganisms suspended in the water samples was determined at the end of the incubation period by employing spectrophotometry at 600 nm, and the residual diesel in the MMC medium was recovered by extraction with petroleum ether and

quantitatively determined by using UV spectrophotometry at 221 nm.

3 RESULTS

3.1 Isolation of Hydrocarbon-Utilizing Strain

A total of three colonies were initially screened from solid mineral medium supplemented with 1% (v/v) sterile diesel as the sole carbon source. After secondary screening, one of the potential strains, isolate SC11-3, showing higher degree of oil degradation rate was selected for further studies. Morphological and biochemical analyses revealed that the isolate SC11-3 was Gram-negative, rod-shaped, and formed cream-yellowish colonies on 2216E agar medium. The isolate exhibited positive results for catalase, urease, gelatin hydrolysis, glucose utilization, motility, nitrate reduction, and OF tests, and could grow at 4°C. Furthermore, the results of 16S rRNA sequencing showed that the isolate SC11-3 was closely related to P. haloplanktis (Blast Max Identity 100%) (Figure 1).

Subsequently, the growth of the isolate SC11-3 was examined at different pH, temperatures, and saline concentrations. After incubation for 24 h with shaking at 150 rpm, the samples were removed to assess the optical density of the growing biomass at 600 nm by using spectrophotometry. The results revealed that the optimum growth temperature and pH of the isolate SC11-3 was 25°C and 8, respectively, and that the adaptive NaCl concentration was in the range of 1–3%. In addition, the isolate also exhibited a relatively stable tolerance to low temperature (<15°C) and high NaCl concentration (>3%) (Figure 2)

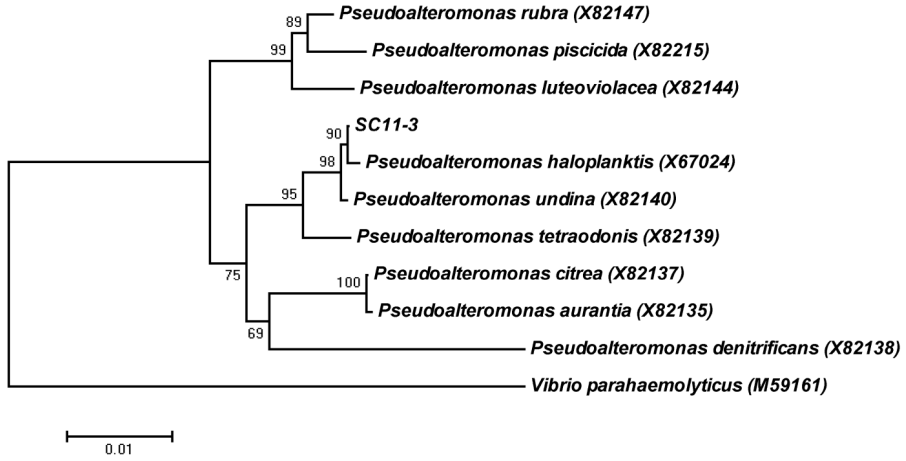

Figure 1: Phylogenetic analysis of 16S rRNA gene sequences of strain SC11-3 and related taxa. The scale bar corresponds to 1% nucleotide sequence difference.

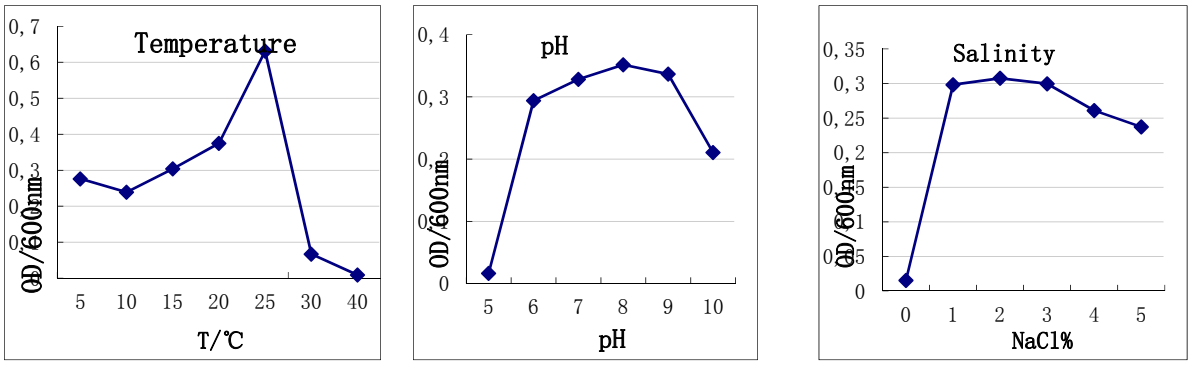

Figure 2: Effects of temperature pH and NaCl concentration on the growth of isolate SC11-3.

Table 1: Morphological and biochemical characteristics of bacterial isolate SC11-3.

Characteristics	*P. haloplanktis* SC11-3
Cell morphology	rod
Colony colour	cream yellowish
Colony diameter	1.5mm
Oxidase	-
Catalase	+
Urease	+
V-P reaction	-
MR-VP reaction	-
Nitrate reduction	+
OF	+
Arginine dehydrolase	-
Gelatin hydrolysis	+
Motility	+
Glucose utilization	+
Citrate utilization	-
TCBS growh	-
O/129 susceptibility	-
Gram staining	-
Temperature (℃)	
5	+
15	+
25	+
30	+
40	-

Note : - negative; + positive.

3.2 Bio-Utilization of Diesel

The diesel degrading capacity of the isolate SC11-3 was investigated by using 2% (v/v) inoculum at different time intervals for up to 15 days. The residual diesel concentration in the medium was observed to decrease with the increasing cultivation time for up to 15 days. To identify whether the bacterial isolate could consume petroleum hydrocarbons as the sole carbon and energy source, diesel was used in this study. As shown in Figure 3, the isolate SC11-3 was able to mineralize diesel as the sole carbon and energy source for growth. The highest bio-utilization capacity was observed on Day 7, whereas no significant difference was found among the degradation rates noted on Day 7 (39.47±8.37%), Day 10 (37.30±7.37%), and Day 15 (37.83±8.84%) (Figure 3). However, the diesel degrading efficiency of the isolate was significantly enhanced when glucose was added as an additional carbon source. Furthermore, the change in the biomass of the isolate SC11-3 was in good agreement with the changes in the degradation rate and glucose concentration. In general, in the presence of glucose, the degradation rate of the isolate was consistently higher than that of the control (Figure 4). The diesel degradation efficiency and growth of the isolate SC11-3 in the MMC medium containing different concentrations of glucose as a co-substrate of carbon source are shown in Figure 4. Both the growth and degradation efficiency of the isolate was restrained at low glucose concentrations. In addition, there was a positive correlation between the degradation efficiency and growth of the isolate in the system (0.5–2 g L-1); however, the correlation differed for 0.5% and 2% inoculum at glucose concentrations of 4–6 and 4–32 g L-1, respectively, as presented in Figure 4(a) (b). Furthermore, for both 0.5% and 2% inoculum, at a glucose concentration of 4 g/L, the biomass decreased with the degradation efficiency remaining relatively high up to approximately 90% after the first appearance of the peaks of degradation efficiency. Then, the growth gradually declined with the increasing concentration of glucose, which may be owing to the inhibitory effect of excessive amount of glucose on the growth of the isolate. Moreover, it was noted that 2.0% inoculum presented relatively more potent tolerance to high concentration of glucose.

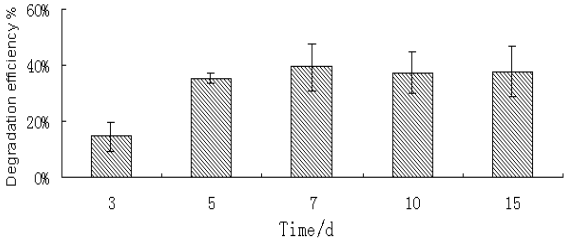

Figure 3: Initial degradation efficiency of strain SC11-3.

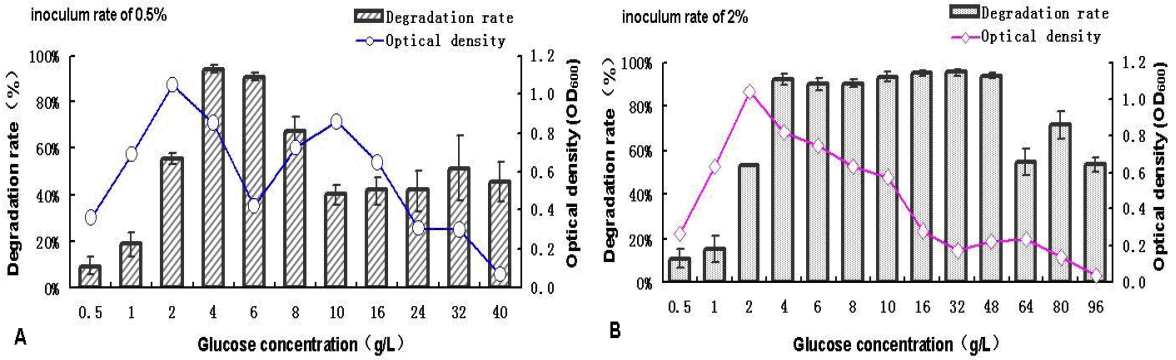

Figure 4. Effects of glucose concentrations on the degradation efficiency and biomass of strain SC11-3 at inoculum rates of 0.5% and 2.0%.

4 DISCUSSIONS

In this study, we reported on a petroleum-degrading strain, *P. haloplanktis* SC11-3, isolated from the gut samples of *P. aibuhitensis*. The limit to higher degradation rate was investigated based on the concentration of glucose added as a supplementary carbon source to the medium. The experimental results indicated that the degree of growth and degradation rate of the isolate SC11-3 varied with the concentration of the glucose supplemented. Furthermore, it was observed that the growth rates of the isolate in the presence of glucose were consistently higher than those of the control, and the degradation rate was significantly improved at a specific glucose concentration. However, with the increase in the amount of glucose added to the medium, the degradation rate declined, which may be due to the inhibitory effect of excessive amount of glucose on bacterial growth. Thus, the above-mentioned results suggest the occurrence of co-metabolism during the process of petroleum degradation by the isolate SC11-3, because glucose can either be consumed by the bacteria as a primary carbon source through direct metabolism or used co-metabolically when bacterial growth requires other non-growth substrates.

Co-metabolism has the advantage of shortening the lag phase in a biotreatment system (Volpe et al. 2009). Recent surveys have revealed that the most important cause for the occurrence of co-metabolism may be the increased activity or amount of microbial biomass (Tittle et al. 1995). Furthermore, it has also been reported that the main reason for the inability of the microorganisms to efficiently degrade PAHs is the lack of catabolic enzyme induction (Wen et al. 2011). Therefore, appropriate co-substrates such as glucose may be useful for the bioremediation of petroleum hydrocarbons because they can promote efficient degradation. In addition, glucose has been reported to stimulate the biodegradation of compost (Jang et al. 2002), and co-metabolism has been extensively applied to many areas of bioremediation (Rentz et al. 2005; Xie et al. 2009). Nevertheless, the applications of co-metabolism in petroleum hydrocarbon degradation are scarcely reported.

A technical limitation in the bioremediation process is the dilution of seeded microorganisms or fertilizers (Radwan et al. 2002; Panchal et al. 2018). As a result, there is an increasing interest in investigating the use of marine sedimentary invertebrates associated with petroleum-utilizing bacteria in bioremediation, which shall provide new efficient ways for cleaning polluted marine environments. These multi-biological systems provide suitable habitats for microorganisms, with carbon source such as glucose, nitrogenous and phosphorus compounds, and vitamins (Radwan and Al-Muteirie 2001). Although the degrading efficiency of *P. haloplanktis* in the present study was observed to be significantly enhanced, more studies will be further performed by our group on in situ bioremediation process on the laboratory scale, such as the degrading efficiency under anoxic conditions as well as association with *Perinereis aibuhitensis* and glucose. The petroleum-utilizing bacterium identified in the present study could be used in bioremediation as a potential candidate for

being artificially immobilized in the digestive tract of worms, which will provide a useful insight into in situ bioremediation of multi-biological systems.

5 CONCLUSIONS

In the present study, Isolate SC11-3 was identified and characterized as a potential hydrocarbon-utilizing microorganisms immobilized in the digestive tract of *Perinereis aibuhitensis*, a marine sedimentary invertebrate with high resistance to pollution. Our study reported the effect of co-metabolism on the activation of petroleum-degrading potential of Isolate SC11-3. Although the mechanism precisely responsible for such effects are generally not known, the current research is starting to unravel the mechanisms for such oil degradation processes. Thus, the findings of bacterial isolate of *P. haloplanktis* here could be used for more detailed future investigations on the oil-degrading genes and environmental factors influencing the bioremediation mechanism. The results of our present study could be helpful in exploring the possibility of cleaning polluted marine environments in a more efficient way and provide new insights into in situ bioremediation of multi-biological systems.

REFERENCES

Atlas R M 1981 Microbial hydrocarbon of petroleum hydrocarbon: an environmental perspective. *Microbiol Rev* **45** 180–209

Balba M T, Al A N, Al D R and Heitzer A 1998 Bioremediation of oil-contaminated soil: microbiological methods for feasibility assessment and field evaluation. *Journal of Microbiological Methods* **32** 155–164

Barois I and Lavelle P 1986 Changes in respiration rate and some physicochemical properties of a tropical soil during transit through Pontoscolex corethrurus (*Glossoscolecidae, Oligochaeta*). *Soil Biology & Biochemistry* **18 (5)** 539-541

Byzov B A, Khomyakov N V, Kharin S A and Kurakov A V 2007 Fate of soil bacteria and fungi in the gut of earthworms. *European Journal of Soil Biology* **43** 149–156.

Chaerun S K, Tazaki K, Asada R and Kogure K 2004 Bioremediation of coastal areas 5 years after the Nakhodka oil spill in the Sea of Japan: isolation and characterization of hydrocarbon-degrading bacteria. *Environment International* **30** 911–922

Chen X, Zhou Y, Yang D, Zhao H, Wang L and Yuan X 2012 CYP4 mRNA expression in marine polychaete *Perinereis aibuhitensis* in response to petroleum hydrocarbon and deltamethrin. *Marine Pollution Bulletin* **64** 1782–1788

Jang J C, Shin P K, Yoon J S, Lee I M, Lee H S and Kim M N 2002 Glucose effect on the biodegradation of plastics by compost from food garbage. *Polymer Degradation and Stability* **76** 155–159

Jørgensen A, Giessing A M B, Rasmussen L J and Andersen O 2008 Biotransformation of polycyclic aromatic hydrocarbons in marine polychaetes. *Marine Environmental Research* **65** 171–186

Knapp B A, Podmirseg S M, Seeber J, Meyer E and Insam H 2009 Diet-related composition of the gut microbiota of Lumbricus rubellus as revealed by a molecular fingerprinting technique and cloning. *Soil Biology & Biochemistry*. **41** 2299–2307

Lavelle P, Rouland C, Diouf M, Binet F and Kersanté A 2005 Regulation of microbial activities by roots and soil invertebrates. *Journal of Bacteriology* **179 (1)** 7856-7864

Lavellea P, Aubert M and Barot S, et al. 2006 Soil invertebrates and ecosystem services. *European Journal of Soil Biology* **42 (11)** S3–S15

Leahy J G and Colwell R R 1990 Microbial degradation of hydrocarbons in the environment. *Microbiol Rev* **54** 305–315

Mikesell M D, Kukor J J and Olsen R H 1993/1994 Metabolic diversity of aromatic hydrocarbon-degrading bacteria from a petroleum-contaminated aquifer. *Biodegradation* **4** 249–259

Nicky B B 2004 Bacteria from Fish and Other Aquatic Animals: A Practical Identification Manual

Nilanjana D and Preethy C 2011 Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview. *Biotechnology Research International* **1** 941810

Ortega-González D K, Martínez-González G and Flores C M, et al. 2015 Amycolatopsis sp. Poz14 isolated from oil-contaminated soil degrades polycyclic aromatic hydrocarbons. *International Biodeterioration & Biodegradation* **99**165-173

Panchal A, Swientoniewski L T and Omarova M, et al. 2018 Bacterial proliferation on clay nanotube Pickering emulsions for oil spill bioremediation. *Colloids & Surfaces B Biointerfaces* **164** 27-33

Radwan S S and Al-Muteirie A S 2001 Vitamin requirements of hydrocarbon-utilizing soil bacteria. *Microbiological Research* **155** 301–307

Radwan S S, Al-Hasan R H, Salamah S and Al-Dabbous S 2002 Bioremediation of oily sea water by bacteria immobilized in biofilms coating macroalgae. *International Biodeterioration & Biodegradation* **50** 55–59

Rentz J A, Alvarez P J J and Schnoor J L 2005 Benzo[a]pyrene co-metabolism in the presence of plant root extracts and exudates: implications for phytoremediation. *Environmental Pollution* **136** 477–

484
Ridgway H F, Safarik J, Phipps D, Carl P and Clark D 1990 Identification and catabolic activity of well-derived gasoline-degrading bacteria from a contaminated aquifer. *Applied and Environmental Microbiology* **56** 3565–3575
Santos J J D and Maranho L T 2018 Rhizospheric microorganisms as a solution for the recovery of soils contaminated by petroleum: A review. *Journal of Environmental Management* **210** 104-113
Tittle P C, Liu Y T, Strand S E and Stensel H D 1995 Use of alternative growth substrates to enhance PAH degradation. In: R. E. Hinchee (eds) *Bioremediation of Recalcitrant Organics* 1–7
Vergeynst L, Wegeberg S and Aamand J, et al. 2018 Biodegradation of marine oil spills in the Arctic with a Greenland perspective. *Science of the Total Environment* **626** 1243-1258
Volpe A, Del M G, Rossetti S, Tandoi V and Lopez A 2009 Enhanced bioremediation of methyl tert-butyl ether (MTBE) by microbial consortia obtained from contaminated aquifer material. *Chemosphere* **75** 149–155
Watanabe K, Teramoto M, Futamata H and Harayama S 1998 Molecular detection, isolation, and physiological characterization of functionally dominant phenol-degrading bacteria in activated sludge. *Applied and Environmental Microbiology* **64** 4396–4402
Wen J W, Gao D W, Zhang B and Liang H 2011 Co-metabolic degradation of pyrene by indigenous white-rot fungus Pseudotrametes gibbosa from the northeast China. *International Biodeterioration & Biodegradation* **65** 600–604
Williams, Wilkins 1986 Bergey's Manual of Systematic Bacteriology. ISBN 0-683-04108-8
Woese C R and Fox G E 1977 Phylogenetic structure of the prokaryotic domain: the primary kingdoms. *Proceedings of the National Academy of Sciences of the United States of America* **74 (11)** 5088–5090
Xie S, Liu J X, Li L and Qiao C L 2009 Biodegradation of malathion by Acinetobacter johnsonii MA19 and optimization of cometabolism substrates. *Journal of Environmental Sciences* **21** 76–82