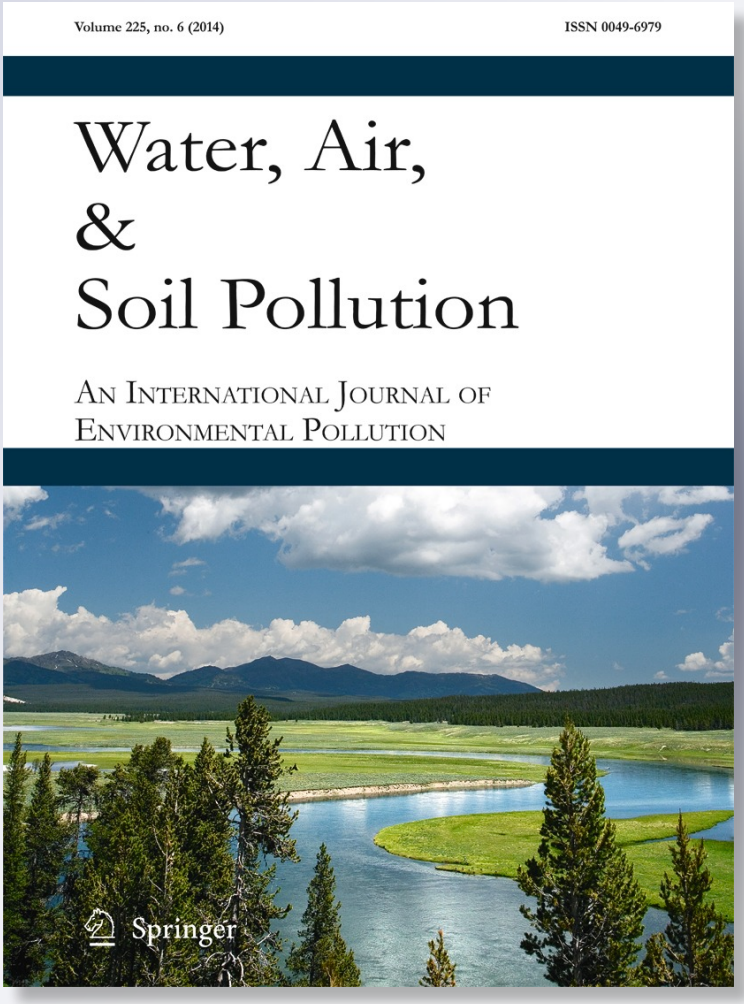
Dominant Indigenous Bacteria in Gasoline-Treated Marshes Around Lake Pontchartrain, Louisiana

Astha Gupta, Kewei Yu & Joong-Wook Park

Water, Air, & Soil Pollution
An International Journal of Environmental Pollution

ISSN 0049-6979
Volume 225
Number 6

Water Air Soil Pollut (2014) 225:1-10
DOI 10.1007/s11270-014-2011-0



Springer

Water Air Soil Pollut (2014) 225:2011
DOI 10.1007/s11270-014-2011-0

Dominant Indigenous Bacteria in Gasoline-Treated Marshes Around Lake Pontchartrain, Louisiana

Astha Gupta · Kewei Yu · Joong-Wook Park

Received: 6 January 2014 / Accepted: 14 May 2014 / Published online: 27 May 2014

Abstract Considerable amount of gasoline from natural and anthropogenic sources, such as urban runoff during hurricanes and oil discharges from pleasure crafts, has been released into Lake Pontchartrain, Louisiana, which poses a threat to the lake marsh ecosystems. In this research, we evaluated the impact of gasoline on indigenous bacterial communities in three types of marsh sediments collected from the Lake Pontchartrain. Our data show that several bacterial species are significantly enriched in gasoline-treated sediments. DNA sequencing data indicate that the enriched bacteria in response to the gasoline treatment are *Acidocella* and *Burkholderia* spp. in freshwater marsh; *Mariprofundus*, *Nitrosospira*, and *Ferrimicrobium* spp. in brackish marsh; and three *Pseudomonas* spp. in salt marsh. Our research will help to understand a gasoline bioremediation by indigenous bacteria and to develop site-specific bioremediation strategies for the Lake Pontchartrain.

Keywords Gasoline · Bioremediation · Microbial community · Lake Pontchartrain

Electronic supplementary material The online version of this article (doi:10.1007/s11270-014-2011-0) contains supplementary material, which is available to authorized users.

A. Gupta · K. Yu · J.-W. Park (✉)
Department of Biological and Environmental Sciences, Troy University,
Troy, AL 36082, USA
e-mail: jwpark@troy.edu

1 Introduction

Marshes are important ecosystems because they harbor terrestrial and aquatic species, provide recreational opportunities, store floodwaters, and influence surface water quality by transforming or storing pollutants. There is, however, a continuing loss of marshes due to coastal developments and contamination problems (Unites States Environmental Protection Agency 2013). Our study focused on marshes around the Lake Pontchartrain, which are vulnerable to gasoline contamination due to the fact that the lake receives high amount of gasoline discharged from pleasure crafts or by urban runoff from roads and highways after hurricanes or heavy rain. In 2005, hurricane Katrina damaged more than 100 oil and gas platforms causing oil spill accidents that released about 8 million gallons of oil (Manuel 2006). The oil-contaminated floodwaters were pumped into the Lake Pontchartrain, which subsequently contaminated the lake and the marshes around the lake (Manuel 2006). Similar accidents are likely to happen due to hurricanes and storms. The estimated annual release of unburned oil and gasoline into the Lake Pontchartrain from the operation of two-cycle engine pleasure crafts is 150,000 gallons per year (Department of Natural Resources and State of Louisiana 1995). Lake Pontchartrain is also susceptible to gasoline contamination due to possible leaks from newly constructed Parkway Pipeline beneath the lake, which transports gasoline and other oils from refineries in Norco, LA, to Collins, MS (Office of the Governor and State of Louisiana 2013). The gasoline contamination poses a

threat to the marshes and living organisms as a long-term effect, especially if a huge amount of gasoline spill is caused by hurricanes, heavy rain, or other natural disasters (Dietrich et al. 2012).

Gasoline, the distillate of crude oil that contains C_4 to C_{12} hydrocarbons (Sawyer 1993), can cause serious environmental problems and has harmful effects on living organisms (Chikere et al. 2011; Militon et al. 2010; Marin et al. 2005; Weidong et al. 2011). Gasoline is thought to be carcinogenic and toxic to living organisms (Jain et al. 2011). Hydrocarbon compounds found in gasoline can persist in soil/sediment particles and cause damage to their physical properties (Militon et al. 2010). Benzene in gasoline (Burri et al. 2004) is a toxic chemical causing aplastic anemia, leukemia, and multiple myeloma (Yardley-Jones et al. 1991). Therefore, effective gasoline cleanup strategies are needed to resolve these problems (Bundy et al. 2002).

The majority of principal hydrocarbon components of gasoline are biodegradable (Prince 2003). Among the plethora of microorganisms in soils, many microorganisms are known to degrade hydrocarbon compounds (Juwarkar et al. 2010). The increasing demand for effective cleaning of polluted environments has led to development and wide use of bioremediation strategies that are cost-effective and eco-friendly and have a potential to detoxify a wide range of oil contaminants (Chikere et al. 2009; da Silva et al. 2009; Liu et al. 2010; Zouboulis and Moussas 2011). Bioaugmentation is one of the popular bioremediation strategies; oil-degrading microorganisms are inoculated into contaminated environments to increase the rate of bioremediation (Mrozik and Piotrowska-Seget 2010). It has been reported that a treatment of gasoline-degrading microorganisms expedites the gasoline biodegradation process in contaminated areas (Liu et al. 2011; Madueño et al. 2011). However, this strategy also has a risk of causing unexpected ecological problems by introducing foreign microorganisms. Because the use of indigenous microorganisms reduces the potential environmental risk, understanding indigenous microbial community in the polluted site is required in order to develop site-specific bioremediation strategies (Grenni et al. 2012; Łebkowska et al. 2011; Madueño et al. 2011; Watanabe 2001).

The ultimate goal of our research is to develop site-specific gasoline bioremediation strategies for the Lake Pontchartrain and adjacent areas. In this study, indigenous marsh bacteria at the lake that thrive in response to gasoline treatment were detected and identified. Our research helps to understand the bioremediation process of gasoline and to identify suitable gasoline-degrading bacteria for site-specific bioremediations of the Lake Pontchartrain.

2 Materials and Methods

2.1 Sampling and Chemical Analysis

Marsh sediment samples were collected along the northeastern side of Lake Pontchartrain on May 6, 2011 (Fig. 1). Three sediment samples were collected from a freshwater marsh (FM), a brackish marsh (BM), and a salt marsh (SM). The salt and brackish marsh sites were primarily vegetated by *Spartina alterniflora* and *Spartina patens*, which are saltwater plants. The freshwater marsh site was covered by *Sagittaria lancifolia*, *Typha angustifolia*, *Potamogeton nodosus*, and *Scirpus olneyi*, most of which are freshwater plants. Salinities of the marshes are shown in Table 1. After removing the surface vegetation, the top 30 cm of the sediment was sampled, transported on ice, and stored at 4 °C before use. Three marsh sediments (FM, BM, and SM) were sent to Central Analytical Instruments Research Laboratory, Louisiana State University (Baton Rouge, LA) for analysis of carbon and nitrogen contents, anions (method EPA 300.0), total phosphorus (method EPA 365.3), and metal (method EPA 200.7) (Tables 1 and S1). All sediment results are in dry weight basis.

2.2 Microcosm Preparation and DNA Extraction

Two sets of microcosms were prepared in triplicate: (1) non-treated control and (2) microcosms treated with 2 % (20 μL of gasoline in 1 g of sediment) of commercial gasoline (dos Santos et al. 2011) obtained from a local gas station (Troy, AL) on February 14, 2012. One gram of sediment was used to set up microcosms in 15-mL glass test tubes. All microcosms were incubated at room temperature for 1 week. Total DNA was extracted from 0.3 g of microcosm sediment samples, both treated and control sediments, using a PowerSoil™ DNA Isolation Kit according to the manufacturer's instructions (MoBio Laboratories, Carlsbad, CA). Extracted DNAs were stored at −20 °C prior to analysis.

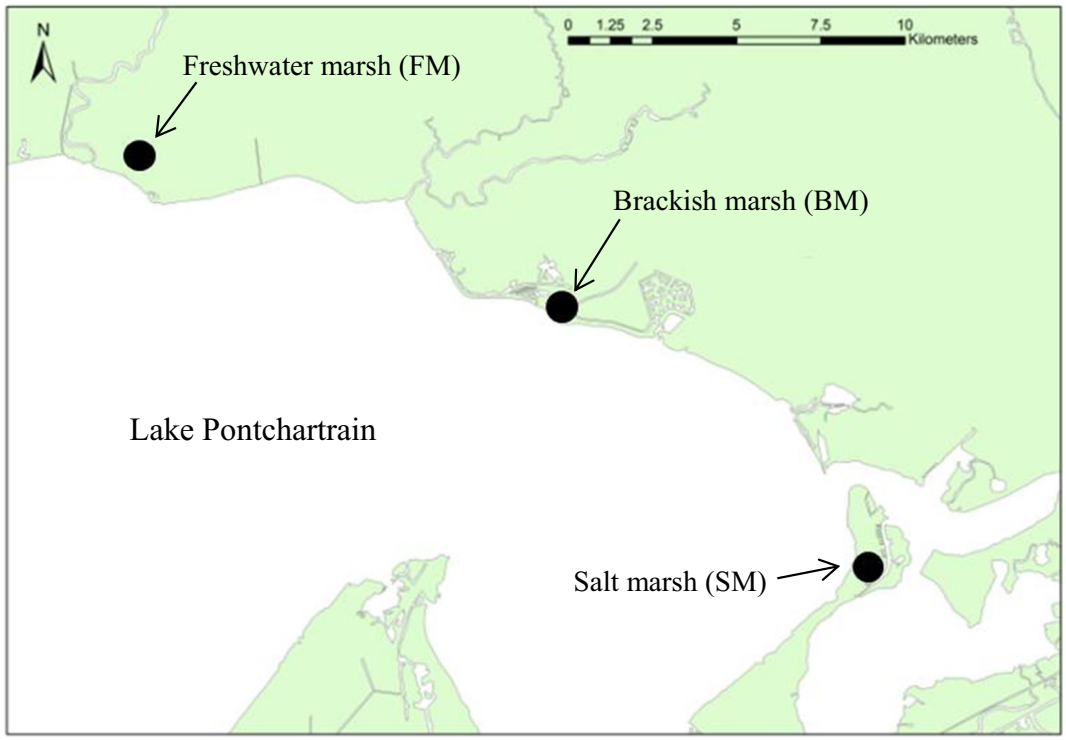


Fig. 1 Location of three sampling sites along the northeastern side of Lake Pontchartrain

2.3 Polymerase Chain Reaction

The V3 region of bacterial 16S ribosomal RNA (rRNA) gene was amplified by polymerase chain reaction (PCR) using the forward primer 341F with GC clamp and reverse primer 534R (Muyzer et al. 1993). PCR mixture was 50 uL, containing 10 pmol of each primer, 1 μL of template DNA, 0.25 mM of dNTP, 5 μL of 10× Green Taq PCR buffer, and 1 U of Green Taq DNA polymerase (GenScript, Piscataway, NJ). The PCR reactions were carried out with a DNA thermal cycler (GeneAmp PCR System 2700, Applied Biosystems, Foster City, CA) at an initial temperature of 95 °C for 5 min, followed by 35 cycles of 95 °C for 20 s, 55 °C for 45 s, and 72 °C for 45 s. A final extension was carried out at 72 °C for 7 min. After amplification, the products were analyzed by electrophoresis in 1.5 % agarose gel. The gels were stained with ethidium bromide and photographed on a UV transilluminator (Fisher Scientific, Pittsburgh, PA).

2.4 Denaturing Gradient Gel Electrophoresis and DNA Sequencing

Amplified bacterial 16S rRNA gene fragments were separated by using a Bio-Rad DCode™ system (Bio-Rad Laboratories, Hercules, CA). All triplicate microcosms were subjected to denaturing gradient gel electrophoresis (DGGE) analysis. Six percent polyacrylamide gels were made with a linear denaturing gradient with 40 % denaturant at the top to 60 % denaturant at the bottom of the gel. The PCR products were loaded onto the polyacrylamide gels in a 1× Tris-acetate-EDTA (TAE, pH 8.0) buffer. The electrophoresis was carried out in a 1× TAE buffer for 16 h at 45 V. The DGGE gel

Table 1 Key property of three marshes at Lake Pontchartrain

	pH	Salinity (ppm)	Iron (mg/kg)	Sulfur (mg/kg)
Freshwater marsh (FM)	6.31	7,241	7,986	5,428
Brackish marsh (BM)	7.31	19,866	12,654	4,253
Salt marsh (SM)	7.06	22,446	19,914	5,305

was stained with ethidium bromide and photographed on a UV transilluminator (Fisher Scientific, Pittsburgh, PA). The DGGE patterns of the gasoline-treated marsh sediments were compared to the control sediments to select the bands that were present only in control or gasoline-treated samples. The DGGE bands of interest were excised with a clean blade and incubated with 50 μL of distilled water at 4 °C overnight. Re-amplification of diffused DNAs was performed with the same primer set and PCR conditions as described above. The purity of the excised bands was confirmed by DGGE. If necessary, the DGGE bands were re-excised and the process of PCR-DGGE was repeated until a single DGGE band was observed. The resulting PCR products were purified with QuickClean II PCR Extraction Kit (GenScript Corporation, NJ) according to the manufacturer's instruction and used as templates for DNA sequencing. Each sample was sequenced with forward and reverse primers separately. The resulting DNA sequences were combined together. If necessary, additional DNA sequencings were performed to clarify ambiguous base pairs. The DNA sequencing was conducted by Genewiz Inc. (Genewiz Inc., South Plainfield, NJ). The basic local alignment search tool (BLAST) was used to search homologous DNA sequences in the GenBank DNA libraries (Altschul et al. 1990). A phylogenetic analysis was constructed by using a Molecular Evolutionary Genetics Analysis (MEGA) version 5 (Tamura et al. 2011). Neighbor-joining phylogenetic trees with 1,000 bootstrap replications were constructed with the maximum composite likelihood model for nucleotide substitutions (Tamura et al. 2011).

3 Results

3.1 Chemical Analysis

Key chemical properties of freshwater, brackish, and salt marsh sediments are shown in Table 1. As expected, the salt marsh has high-salinity content, while the freshwater marsh has low-salinity content. All three marsh samples have considerable amounts of iron and sulfur (Table 1).

3.2 DGGE Analysis

The effects of gasoline treatments on microbial community structure were observed using a DGGE analysis (Fig. 2). All triplicate microcosms were analyzed, which showed the same patterns (data not shown). Each of the marsh sediments showed its unique DGGE pattern, and no dominant DGGE band was present in two or more different marshes. One major band in control brackish marsh (BM1), three major bands in gasoline-treated brackish marsh (BM2, BM3, and BM4), two major bands in gasoline-treated freshwater marsh (FM1 and FM2), and three major bands in gasoline-treated salt marsh (SM1, SM2, and SM3) were selected for DNA sequencing. These bands, except BM1, represent bacteria that grow well in the gasoline-treated sediments, not in the control sediments (Fig. 2).

3.3 16S rRNA Gene Sequencing and Phylogenetic Analysis

The selected nine DGGE bands (Fig. 2) were sequenced, and the sequence of each band was compared to homologous sequences in GenBank by the BLAST search. The closest relatives in the GenBank are shown in Table 2. Phylogenetic tree was constructed with the obtained sequences and their close relatives (Fig. 3).

4 Discussion

We showed that the bacterial communities in three different Lake Pontchartrain marshes were greatly changed in response to the gasoline treatment. Even though the three sampling sites are geographically close (Fig. 1), their bacterial communities are significantly different (Fig. 2). These data suggest that one type of microorganism may not be effective to bioremediate all three geographical areas of the Lake Pontchartrain. As a rule of thumb, the visible bands representing individual bacterial species are not produced on DGGE gels unless the population densities represent 1 % of the total bacterial population (Park and Crowley 2005). No high-intensity DGGE band (such as bands BM2, BM3, FM1, FM2, and SM2) was observed in three control sediments (Fig. 2), which represents that sediments originally contain diverse bacterial population that are more or less evenly abundant without dominant species (Smalla et al. 2001). While high-intensity DGGE bands were observed in sediments treated with gasoline (Fig. 2), it suggests that certain indigenous bacteria thrive in response to the gasoline treatment. This is expected because the bacterial community structure tends to change

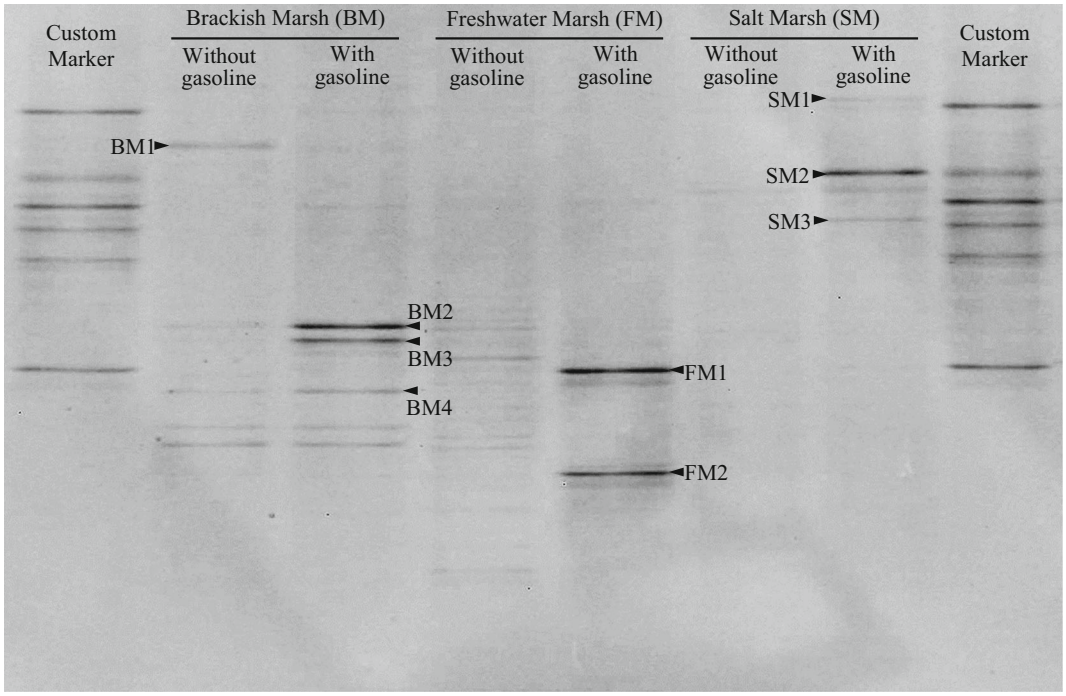


Fig. 2 Bacterial community shift after 2 % of gasoline treatment. DGGE profiles of 16S rRNA gene fragments were analyzed after 1 week of incubation at room temperature. All triplicate microcosms were analyzed, which showed the same pattern. *FM* freshwater marsh, *BM* brackish marsh, *SM* salt marsh

when new energy sources are provided. Several studies have confirmed the change in microbial community structure after input of oil that acts as an energy source (Hazen et al. 2010; Hollaway et al. 1980; Kostka et al. 2011; Lu et al. 2011; MacNaughton et al. 1999; Maruyama et al. 2003; Pucci et al. 2000). Besides oil-degrading bacteria, bacteria resistant to hydrocarbon toxicity or bacteria that can utilize the intermediates produced by oil-degrading bacteria may thrive together (Ekpo and Ebeagwu 2009).

In order to further understand the functional identity of oil degrading bacteria, stable isotope probing (SIP) with ^{13}C-labeled gasoline hydrocarbons is an appropriate tool. In previous research, SIP method has been frequently used to identify microorganisms that degrade hydrocarbons. *Desulfosporosinus* phylotypes (Sun et al. 2014; Winderl et al. 2010a, b), a member of genus *Desulfuromonas* (Kim et al. 2014), five different phylotypes (Sun and Cupples 2012), an uncultured bacterium closely related to *Desulfocapsa* (Bombach et al. 2010),

Table 2 Closest relatives of major DGGE bands

Band	Closest relative found in BLAST	GenBank account #	% Identity
FM1	*Burkholderia* sp. clone	AB558203	80.3
FM2	*Acidocella* sp. clone	EF087979	98.6
BM1	*Thiobacillus prosperus* strain DSM 5130	EU653291	99.4
BM2	*Mariprofundus* sp. GSB2	HQ206653	94.9
BM3	*Nitrosospira* sp. isolate AF	X84658	94.9
BM4	*Ferrimicrobium* sp. clone C.la-6	JX504890	93.5
SM1	*Pseudomonas* sp. LYBRD3-7	HM246142	98.9
SM2	*Pseudomonas* sp. LYBRD3-7	HM246142	100
SM3	*Pseudomonas* sp. CC-OPY-1^T	JQ277453	96.9

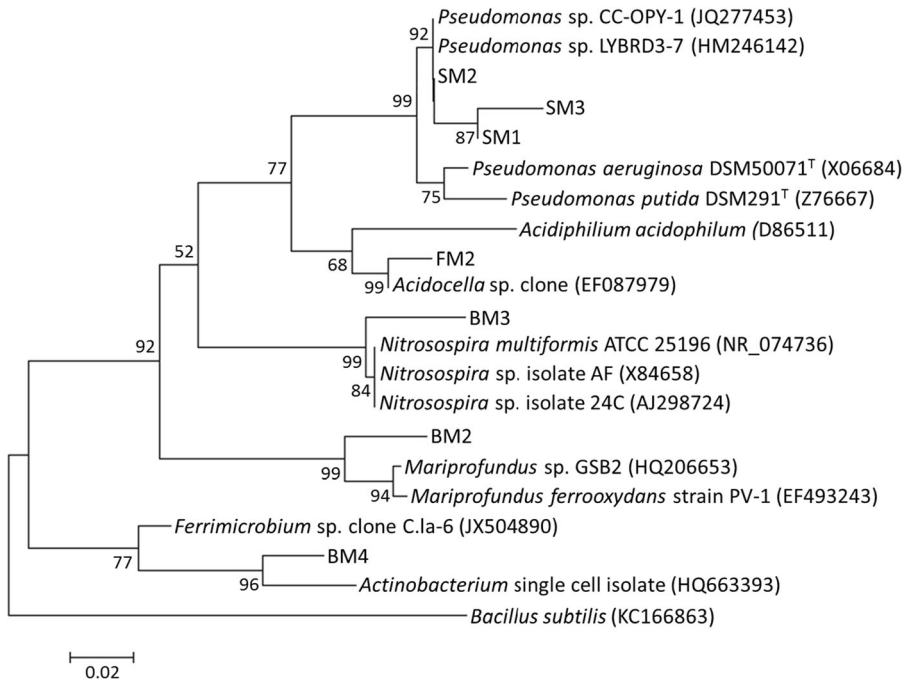


Fig. 3 Neighbor-joining tree based on DNA sequences of seven DGGE bands and related 16S rRNA genes. Excluding primer regions, 153 bp of DNA sequences was used for analysis. Bootstrap values (1,000 replicates) higher than 50 % are indicated at the branch points. The tree was derived from variable region 3 of the 16S rRNA gene. The 16S rRNA genes of *Bacillus subtilis* were used as an outgroup. *FM* freshwater marsh, *BM* brackish marsh, *SM* salt marsh

and a member of genus *Polaromonas* (Sun et al. 2010) were shown to degrade ^{13}C-labeled toluene under various conditions. Similarly, the SIP experiments were used to show benzene degradation by members of *Cryptanaerobacter*/*Pelotomaculum* (Herrmann et al. 2010), *Clostridia* (Kunapuli et al. 2007), and an uncultured microorganism related to clone SB-21 (Oka et al. 2008). Thus, the SIP of ^{13}C-labeled gasoline hydrocarbons has a great potential to extend this research.

In the freshwater marsh microcosms, two DGGE bands were enriched in the gasoline-treated sediments (Fig. 2), and their DNA sequences showed that band FM1 is 80.3 % identical with a *Burkholderia* sp. clone (AB558203) and that band FM2 is 98.6 % identical with a *Acidocella* sp. clone (EF087979) (Table 2). Many *Burkholderia* spp. are well-known hydrocarbon degraders that degrade phenanthrene and other polycyclic aromatic hydrocarbons (PAHs) (Huang et al. 2008; Laurie and Lloyd-Jones 1999; Balashova et al. 1999). *Acidocella* spp. are also known to degrade naphthalene, the simplest PAH, as a sole energy source (Dore et al. 2003) and was found in acidic soils contaminated with crude oil (Hamamura et al. 2005; Röling et al. 2006). Hamamura et al. (2005) also identified *Acidiphilium acidophilum* (D86511) as one of the dominant bacteria in hydrocarbon-contaminated soil (Hamamura et al. 2005).

The diverse bacterial species were observed in brackish marsh sediments with or without gasoline treatments (Fig. 2). Band BM1 was detected only in control sediments, which suggests that the bacterial species representing band BM1 cannot thrive with gasoline. DNA sequencing data show the band BM1 bacteria is phylogenetically close to *Thiobacillus prosperus* strain DSM 5130 (EU653291) (Table 2). *T. prosperus* is a halotolerant metal-oxidizing Betaproteobacterium, which can use iron and sulfur as its energy sources (Huber and Stetter 1989). The brackish marsh used in this study has considerable amounts of iron and sulfur (Table 1), which support that the halotolerant metal-oxidizing bacteria become a dominant species in the control brackish marsh. The three major DGGE bands in gasoline-treated brackish marshes are BM2, BM3, and BM4, which are phylogenetically close to *Mariprofundus* sp. GSB2 (HQ206653), *Nitrosospira* sp. isolate AF (X84658), and *Ferrimicrobium* sp. clone C.la-6 (JX504890), respectively (Table 2). To our best knowledge, gasoline biodegradation by *Mariprofundus*

and *Ferrimicrobium* spp. has not been reported previously. *Mariprofundus* spp., such as *Mariprofundus ferrooxydans* PV-1, are chemolithotrophs that acquire their energy from iron oxidation at microaerophilic environments (Nakagawa and Takai 2008; Singer et al. 2011; Summers et al. 2013). *Ferrimicrobium* spp. are recently discovered acidophilic bacteria that belong to phylum Actinobacteria and reduce iron in anaerobic environments (Johnson et al. 2009). According to Powers et al. (2007), the growth and abundance of some iron-oxidizing bacteria are enhanced by presence of oil (Powers et al. 2007). The results from other studies reveal that *M. ferooxydans* PV-1 and/or GSB2 occupy more than 1 % of the microbial community in an oil-contaminated soil (McBeth et al. 2011; Summer 2012). Ekpo and Ebeagwu (2009) claimed that anaerobic condition may have been created by the introduction of oil to the soil, which favors microaerophilic and anaerobic bacteria, such as *Mariprofundus* and *Ferrimicrobium* spp., to thrive in gasoline-treated iron-rich marshes. The closest phylogenetic relative of band BM3 belongs to the *Nitrosospira* spp., a chemolithoautotrophic ammonia-oxidizing bacteria (Laanbroek et al. 2012). This bacteria carries out ammonia oxidation with an enzyme called ammonia monooxygenase (Kurola et al. 2005a, b; Norton et al. 2002). A number of studies have shown that ammonia-oxidizing bacteria can also oxidize various hydrocarbons by the action of the same enzyme (Deni and Penninckx 1999; Hyman et al. 1988; Keener and Arp 1994; Vannelli et al. 1990). *Nitrosospira multiformis* strains 24C and ATCC 25196 (Fig. 3) have copies of *amo* gene that codes for ammonia monooxygenases (Norton et al. 2002). Kurola et al. reported abundant *Nitrsospira* spp. in the soil having high levels of hydrocarbons (Kurola et al. 2005a, b).

After gasoline treatment to the salt marsh sediments, three dominant DGGE bands appeared (Fig. 2) and DNA sequencing data showed that they all belong to the genus *Pseudomonas* (Table 2). The *Pseudomonas* sp. strain CC-OPY-1^T (Fig. 3) was isolated from an oil-contaminated site in Taiwan (Lin et al. 2012). *Pseudomonas* spp. are well-known hydrocarbon degraders. *Pseudomonas aeruginosa* can degrade a variety of hydrocarbons in oil as its sole carbon source (Belhaj et al. 2002). Many studies have shown the high capacity of oil hydrocarbon degradation by *P. aeruginosa* (Das and Mukherjee 2007; Husain 2008; Karamalidis et al. 2010; Shailubhai et al. 1985; Speight and Arjoon 2012; Yeung et al. 2013; Zhang et al. 2011; Zhang et al. 2012). Similarly, *Pseudomonas putida* is also capable of degrading oil hydrocarbons (Hosokawa et al. 2010). Another factor to consider is that many *Pseudomonas* spp. are able to survive in saline environments. *P. aeruginosa* was isolated in the open ocean (Khan et al. 2007), and benzene, toluene, and xylene (BTX) degrading *Pseudomonas* spp. were isolated from the Pacific Ocean sediment and nearshore surface water (Wang et al. 2008). Based on previous and our data, *Pseudomonas* spp. can be excellent candidates to bioremediate oil-contaminated ocean sediments. One more point that needs to be considered here is that no iron-oxidizing bacteria were identified in the salt marsh sediments, while the salt marsh sediments contain higher iron concentration than the brackish marsh sediments (Table 1). This may be due to a reason that high salinity is toxic to iron-oxidizing bacteria (Cameron et al. 1984). According to Cameron et al. (1984), iron-oxidizing bacteria that were obtained from brackish water were completely inhibited by high amount of salt. Further research is needed to clarify this possible link.

5 Conclusion

Indigenous bacteria that thrive in response to the gasoline treatment were detected in three types of marshes at Lake Pontchartrain (Fig. 1). The bacterial community structure of each marsh was completely different (Fig. 2), and bacteria found in gasoline-enriched sediments, excluding *Mariprofundus* and *Ferrimicrobium* spp., were phylogenetically close to known oil-degrading bacteria (Fig. 3). Understanding indigenous bacteria and their distribution with regard to salinity can be the first step to develop effective site-specific bioremediation strategies for the Lake Pontchartrain without potential environmental risk caused by introducing foreign oil-degrading bacteria.

Acknowledgments This research was funded by the Faculty Development Funds and the Summer Research Grant, Troy University, Troy, AL.

References

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology, 215*(3), 403–410.

Balashova, N. V., Kosheleva, I. A., Golovchenko, N. P., & Boronin, A. M. (1999). Phenanthrene metabolism by *Pseudomonas* and *Burkholderia* strains. *Process Biochemistry, 35*, 291–296.

Belhaj, A., Desnoues, N., & Elmerich, C. (2002). Alkane biodegradation in *Pseudomonas aeruginosa* strains isolated from a polluted zone: identification of *alk*B and *alk*B-related genes. *Research in Microbiology, 153*(6), 339–344.

Bombach, P., Chatzinotas, A., Neu, T. R., Kästner, M., Lueders, T., & Vogt, C. (2010). Enrichment and characterization of a sulfate-reducing toluene-degrading microbial consortium by combining in situ microcosms and stable isotope probing techniques. *FEMS Microbiology Ecology, 71*(2), 237–246.

Bundy, J. G., Paton, G. I., & Campbell, C. D. (2002). Microbial communities in different soil types do not converge after diesel contamination. *Journal of Applied Microbiology, 92*(2), 276–288.

Burri, J., Crockett, R., Hany, R., & Rentsch, D. (2004). Gasoline composition determined by H NMR spectroscopy. *Fuel, 83*, 187–193.

Cameron, F. J., Jones, M. V., & Edwards, C. (1984). Effects of salinity on bacterial iron oxidation. *Current Microbiology, 10*(6), 353–356.

Chikere, C. B., Okpokwasili, G. C., & Chikere, B. O. (2009). Bacterial diversity in a tropical crude oil-polluted soil undergoing bioremediation. *African Journal of Biotechnology, 8*(11), 2535–2540.

Chikere, C. B., Okpokwasili, G. C., & Chikere, B. O. (2011). Monitoring of microbial hydrocarbon remediation in the soil.*3. Biotech, 1*(3), 117–138.

Da Silva, A. C., de Oliveira, F. J. S., Bernardes, D. S., & de França, F. P. (2009). Bioremediation of marine sediments impacted by petroleum. *Applied Biochemistry and Biotechnology, 153*, 58–66.

Das, K., & Mukherjee, A. K. (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.

Deni, J., & Penninckx, M. J. (1999). Nitrification and autotrophic nitrifying bacteria in a hydrocarbon-polluted soil. *Applied and Environmental Microbiology, 65*(9), 4008–4013.

Department of Natural Resources, State of Louisiana. (1995). Sources and impacts of pollution in Lake Pontchartrain. http://dnr.louisiana.gov/index.cfm?md=pagebuilder&tmp=home&pid=269. Accessed 1 January 2014.

Dietrich, J. C., Trahan, C. J., Howard, M. T., Fleming, J. G., Weaver, R. J., Tanaka, S., et al. (2012). Surface trajectories of oil transport along the northern coastline of the Gulf of Mexico. *Continental Shelf Research, 41*, 17–47.

Dore, S. Y., Clancy, Q. E., Rylee, S. M., & Kulpa, C. F. (2003). Naphthalene-utilizing and mercury-resistant bacteria isolated from an acidic environment. *Applied Microbiology and Biotechnology, 63*(2), 194–199.

Dos Santos, H. F., Cury, J. C., do Carmo, F. L., dos Santos, A. L., Tiedje, J., van Elsas, J. D., et al. (2011). Mangrove bacterial diversity and the impact of oil contamination revealed by pyrosequencing: Bacterial proxies for oil pollution. *PLoS ONE, 6*(3), e16943.

Ekpo, M. A., & Ebeagwu, C. J. (2009). The effect of crude oil on microorganisms and dry matter of fluted pumpkin (*Telfairiaoccidentalis*). *Scientific Research and Essays, 4*(8), 733–739.

Grenni, P., Falconi, F., & Caracciolo, A. B. (2012). Microcosm experiments for evaluating natural bioremediation of contaminated ecosystems. *Chemical Engineering, 28*, 7–12.

Hamamura, N., Olson, S. H., Ward, D. M., & Inskeep, W. P. (2005). Diversity and functional analysis of bacterial communities associated with natural hydrocarbon seeps in acidic soils at Rainbow Springs, Yellowstone National Park. *Applied and Environmental Microbiology, 71*(10), 5943–5950.

Hazen, T. C., Dubinsky, E. A., DeSantis, T. Z., Andersen, G. L., Piceno, Y. M., Singh, N., et al. (2010). Deep-sea oil plume enriches indigenous oil-degrading bacteria. *Science, 330*(6001), 204–208.

Herrmann, S., Kleinsteuber, S., Chatzinotas, A., Kuppardt, S., Lueders, T., Richnow, H.-H., et al. (2010). Functional characterization of an anaerobic benzene-degrading enrichment culture by DNA stable isotope probing. *Environmental Microbiology, 12*(2), 401–411.

Hollaway, S. L., Faw, G. M., & Sizemore, R. K. (1980). The bacterial community composition of an active oil field in the northwestern Gulf of Mexico. *Marine Pollution Bulletin, 11*(6), 153–156.

Hosokawa, R., Sakaguchi, N., & Okuyama, H. (2010). Establishment and characterization of turbine oil-degrading bacterial consortia. *International Biodeterioration and Biodegradation, 64*(6), 519–524.

Huang, X., Tian, Y., Luo, Y. R., Liu, H. J., Zheng, W., & Zheng, T. L. (2008). Modified sublimation to isolate phenanthrene-degrading bacteria of the genera *Sphingomonas* and *Burkholderia* from Xiamen oil port. *Marine Pollution Bulletin, 57*, 538–543.

Huber, H., & Stetter, K. O. (1989). *Thiobacillus prosperus* sp. nov., represents a new group of halotolerant metal-mobilizing bacteria isolated from a marine geothermal field. *Archives of Microbiology, 151*(6), 479–485.

Husain, S. (2008). Literature overview: microbial metabolism of high molecular weight polycyclic aromatic hydrocarbons. *Remediation Journal, 18*(2), 131–161.

Hyman, M. R., Murton, I. B., & Arp, D. J. (1988). Interaction of ammonia monooxygenase from *Nitrosomonaseuropaea* with alkanes, alkenes, and alkynes. *Applied and Environmental Microbiology, 54*(12), 3187–3190.

Jain, P. K., Gupta, V. K., Gaur, R. K., Lowry, M., Jaroli, D. P., & Chauhan, U. K. (2011). Bioremediation of petroleum oil contaminated soil and water. *Research Journal of Environmental Toxicology, 5*(1), 1–26.

Johnson, D. B., Bacelar-Nicolau, P., Okibe, N., Thomas, A., & Hallberg, K. B. (2009). *Ferrimicrobium acidiphilum* gen. nov., sp. nov. and *Ferrithrixthermotolerans*gen. nov., sp. nov.: Heterotrophic, iron-oxidizing, extremely acidophilic actinobacteria. *International Journal of Systematic and Evolutionary Microbiology, 59*(5), 1082–1089.

Juwarkar, A. A., Singh, S. K., & Mudhoo, A. (2010). A comprehensive overview of elements in bioremediation. *Reviews in Environmental Science and Biotechnology, 9*(3), 215–288.

Karamalidis, A. K., Evangelou, A. C., Karabika, E., Koukkou, A. I., Drainas, C., & Voudrias, E. A. (2010). Laboratory scale bioremediation of petroleum-contaminated soil by indigenous microorganisms and added *Pseudomonas aeruginosa* strain Spet. *Bioresource Technology, 101*(16), 6545–6552.

Keener, W. K., & Arp, D. J. (1994). Transformations of aromatic compounds by *Nitrosomonaseuropaea*. *Applied and Environmental Microbiology, 60*(6), 1914–1920.

Khan, N. H., Ishii, Y., Kimata-Kino, N., Esaki, H., Nishino, T., Nishimura, M., et al. (2007). Isolation of Pseudomonas aeruginosa from open ocean and comparison with freshwater, clinical, and animal isolates. *Microbial Ecology, 53*(2), 173–186.

Kim, S.-J., Park, S.-J., Cha, I.-T., Min, D., Kim, J.-S., Chung, W.-H., et al. (2014). Metabolic versatility of toluene-degrading, iron-reducing bacteria in tidal flat sediment, characterized by stable isotope probing-based metagenomic analysis. *Environmental Microbiology, 16*(1), 189–204.

Kostka, J. E., Prakash, O., Overholt, W. A., Green, S. J., Freyer, G., Canion, A., et al. (2011). Hydrocarbon-degrading bacteria and the bacterial community response in Gulf of Mexico beach sands impacted by the Deepwater Horizon oil spill. *Applied and Environmental Microbiology, 77*(22), 7962–7974.

Kunapuli, U., Lueders, T., & Meckenstock, R. U. (2007). The use of stable isotope probing to identify key iron-reducing microorganisms involved in anaerobic benzene degradation. *The ISME Journal, 1*(7), 643–653.

Kurola, J., Wittmann, C., Salkinoja-Salonen, M., Aarnio, T., & Romantschuk, M. (2005a). Application of cation-exchange membranes for characterisation and imaging ammonia-oxidising bacteria in soils. *FEMS Microbiology Ecology, 53*(3), 463–472.

Kurola, J., Salkinoja-Salonen, M., Aarnio, T., Hultman, J., & Romantschuk, M. (2005b). Activity, diversity and population size of ammonia-oxidising bacteria in oil-contaminated landfarming soil. *FEMS Microbiology Letters, 250*(1), 33–38.

Laanbroek, H. J., Keijzer, R. M., Verhoeven, J. T. A., & Whigham, D. F. (2012). The distribution of ammonia-oxidizing betaproteobacteria in stands of Black Mangroves (*Avicenniagerminans*). *Frontiers in Microbiology, 3*(153), 1–11.

Laurie, A. D., & Lloyd-Jones, G. (1999). Conserved and hybrid meta-cleavage operons from PAH-degrading *Burkholderia* RP007. *Biochemical and Biophysical Research Communications, 262*(1), 308–314.

Łebkowska, M., Zborowska, E., Karwowska, E., Miaśkiewicz-Pęska, E., Muszyński, A., Tabernacka, A., et al. (2011). Bioremediation of soil polluted with fuels by sequential multiple injection of native microorganisms: *field-scale processes in Poland. Ecological Engineering, 37*(11), 1895–1900.

Lin, S. Y., Hameed, A., Liu, Y. C., Hsu, Y. H., Lai, W. A., Chen, W. M., et al. (2012). *Pseudomonas sagittaria* sp. nov., a novel siderophore-producing bacterium isolated from oil-contaminated soil. *International Journal of Systematic and Evolutionary Microbiology, 63*(7), 2410–2417.

Liu, W., Luo, Y., Teng, Y., Li, Z., & Ma, L. Q. (2010). Bioremediation of oily sludge-contaminated soil by stimulating indigenous microbes. *Environmental Geochemistry and Health, 32*(1), 23–29.

Liu, P. W. G., Chang, T. C., Whang, L. M., Kao, C. H., Pan, P. T., & Cheng, S. S. (2011). Bioremediation of petroleum hydrocarbon contaminated soil: effects of strategies and microbial community shift. *International Biodeterioration and Biodegradation, 65*(8), 1119–1127.

Lu, Z., Deng, Y., Nostrand, J. D. V., He, Z., Voordeckers, J., Zhou, A., et al. (2011). Microbial gene functions enriched in the deepwater horizon deep-sea oil plume. *The ISME Journal, 6*(2), 451–460.

MacNaughton, S. J., Stephen, J. R., Venosa, A. D., Davis, G. A., Chang, Y. J., & White, D. C. (1999). Microbial population changes during bioremediation of an experimental oil spill. *Applied and Environmental Microbiology, 65*(8), 3566–3574.

Madueño, L., Coppotelli, B. M., Alvarez, H. M., & Morelli, I. S. (2011). Isolation and characterization of indigenous soil bacteria for bioaugmentation of PAH contaminated soil of semi-arid Patagonia, Argentina. *International Biodeterioration and Biodegradation, 65*(2), 345–351.

Manuel, J. (2006). In Katrina's wake. *Environmental Health Perspectives, 114*(1), A32–A39.

Marin, J. A., Hernandez, T., & Garcia, C. (2005). Bioremediation of oil refinery sludge by land farming in semiarid conditions: influence on soil microbial activity. *Environmental Research, 98*(2), 185–195.

Maruyama, A., Ishiwata, H., Kitamura, K., Sunamura, M., Fujita, T., Matsuo, M., et al. (2003). Dynamics of microbial populations and strong selection for *Cycloclasticus pugetii* following the Nakhodka oil spill. *Microbial Ecology, 46*(4), 442–453.

McBeth, J. M., Little, B. J., Ray, R. I., Farrar, K. M., & Emerson, D. (2011). Neutrophilic iron-oxidizing "zetaproteobacteria" and mild steel corrosion in nearshore marine environments. *Applied and Environmental Microbiology, 77*(4), 1405–1412.

Militon, C., Boucher, D., Vachelard, C., Perchet, G., Barra, V., Troquet, J., et al. (2010). Bacterial community changes during bioremediation of aliphatic hydrocarbon-contaminated soil. *FEMS Microbiology Ecology, 74*(3), 669–681.

Mrozik, A., & Piotrowska-Seget, Z. (2010). Bioaugmentation as a strategy for cleaning up of soils contaminated with aromatic compounds. *Microbiological Research, 165*(5), 363–375.

Muyzer, G., de Waal, E. C., & Uitterlinden, A. G. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16SrRNA. *Applied and Environmental Microbiology, 59*, 695–700.

Nakagawa, S., & Takai, K. (2008). Deep-sea vent chemoautotrophs: diversity, biochemistry and ecological significance. *FEMS Microbiology Ecology, 65*(1), 1–14.

Norton, J. M., Alzerreca, J. J., Suwa, Y., & Klotz, M. G. (2002). Diversity of ammonia monooxygenase operon in autotrophic ammonia-oxidizing bacteria. *Archives of Microbiology, 177*(2), 139–149.

Office of the Governor, State of Louisiana. (2013). Governor Jindal and Parkway Pipeline announce upcoming completion of $230 million energy pipeline project. http://gov.la.gov/index.cfm?md=newsroom&tmp=detail&catID=2&articleID=4196&navID=3.Accessed 1 January 2014.

Oka, A. R., Phelps, C. D., McGuinness, L. M., Mumford, A., Young, L. Y., & Kerkhof, L. J. (2008). Identification of critical members in a sulfidogenic benzene-degrading consortium by DNA stable isotope probing. *Applied and Environmental Microbiology, 74*(20), 6476–6480.

Park, J. W., & Crowley, D. E. (2005). Normalization of soil DNA extraction for accurate quantification of target genes by real-time PCR and DGGE. *Biotechniques, 38*(4), 579–586.

Powers, J. P., Corwin, A. B., Schmall, P. C., & Kaeck, W. E. (2007). Groundwater chemistry, bacteriology, and fouling of dewatering systems. In J. P. Powers (Ed.), *Construction dewatering and groundwater control* (pp. 195–221). New Jersey: John Wiley & Sons, Inc.

Prince, R. C. (2003). *Petroleum and other hydrocarbons, biodegradation of. Encyclopedia of Environmental Microbiology.* New Jersey: John Wiley.

Pucci, O. H., Bak, M. A., Peressutti, S. R., Klein, I., Härtig, C., Alvarez, H. M., et al. (2000). Influence of crude oil contamination on the bacterial community of semiarid soils of Patagonia (Argentina). *Acta Biotechnologica, 20*(2), 129–146.

Röling, W. F. M., Ortega-Lucach, S., Larter, S. R., & Head, I. M. (2006). Acidophilic microbial communities associated with a natural, biodegraded hydrocarbon seepage. *Journal of Applied Microbiology, 101*(2), 290–299.

Sawyer, R. F. (1993). Trends in auto emissions and gasoline composition. *Environmental Health Perspectives Supplements, 101*(6), 5–12.

Shailubhai, K., Rao, N. N., & Modi, V. V. (1985). Degradation of petroleum industry oil sludge by *Rhodotorularubra* and *Pseudomonas aeruginosa*. *Oil and Petrochemical Pollution, 2*(2), 133–136.

Singer, E., Emerson, D., Webb, E. A., Barco, R. A., Kuenen, J. G., Nelson, W. C., et al. (2011). *Mariprofundus ferrooxydans* PV-1 the first genome of a marine Fe(II) oxidizing *Zetaproteobacterium. PLoS ONE, 6*(9), e25386.

Smalla, K., Wieland, G., Buchner, A., Zock, A., Parzy, J., Kaiser, S., et al. (2001). Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. *Applied and Environmental Microbiology, 67*(10), 4742–4751.

Speight, J. G., & Arjoon, K. K. (2012). Biodegradation of petroleum. In J. G. Speight (Ed.), *Bioremediation of Petroleum and Petroleum Products* (pp. 305–359). Massachusetts: Wiley-Scrivener.

Summer, N. (2012). Bacteriophage replacement of chemicals biocides in deepwater pipelines and reservoirs: a proof of concept study. http://www.netl.doe.gov/technologies/oil-gas/publications/EPact/08121-2902-04-final-report.pdf. Accessed 1 January 2014.

Summers, Z. M., Gralnick, J. A., & Bond, D. R. (2013). Cultivation of an obligate Fe(II)-oxidizing lithoautotrophic bacterium using electrodes. *MBio, 4*(1), e00420–12.

Sun, W., & Cupples, A. M. (2012). Diversity of five anaerobic toluene-degrading microbial communities investigated using stable isotope probing. *Applied and Environmental Microbiology, 74*(4), 972–980.

Sun, W., Xie, S., Luo, C., & Cupples, A. M. (2010). Direct link between toluene degradation in contaminated-site microcosms and a *Polaromonas* strain. *Applied and Environmental Microbiology, 76*(3), 956–959.

Sun, W., Sun, X., & Cupples, A. M. (2014). Identification of *Desulfosoporosinus* as toluene-assimilating microorganisms from a methanogenic consortium. *International Biodeterioration and Biodegradation, 88*, 13–19.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution, 28*(10), 2731–2739.

Unites States Environmental Protection Agency. (2013). Marshes. http://water.epa.gov/type/wetlands/marsh.cfm. Accessed 1 January 2014.

Vannelli, T., Logan, M., Arciero, D. M., & Hooper, A. B. (1990). Degradation of halogenated aliphatic compounds by the ammonia- oxidizing bacterium *Nitrosomonaseuropaea. Applied and Environmental Microbiology, 56*(4), 1169–1171.

Wang, L., Qiao, N., Sun, F., & Shao, Z. (2008). Isolation, gene detection and solvent tolerance of benzene, toluene and xylene degrading bacteria from nearshore surface water and Pacific Ocean sediment. *Extremophiles, 12*(3), 335–342.

Watanabe, K. (2001). Microorganisms relevant to bioremediation. *Current Opinion in Biotechnology, 12*(3), 237–241.

Weidong, D., Yunyang, W., Ningning, Z., Jiajia, F., Zhihuan, Z., Lijun, C., et al. (2011). Status quo of soil petroleum contamination and evolution of bioremediation. *Petroleum Science, 8*(4), 502–514.

Winderl, C., Penning, H., von Netzer, F., Meckenstock, R. U., & Lueders, T. (2010). DNA-SIP identifies sulfate-reducing Clostridia as important toluene degraders in tar-oil-contaminated aquifer sediment. *The ISME Journal, 4*(10), 1314–1325.

Yardley-Jones, A., Anderson, D., & Parke, D. V. (1991). The toxicity of benzene and its metabolism and molecular pathology in human risk assessment. *British Journal of Industrial Medicine, 48*, 437–444.

Yeung, C. W., Stempvoort, D. R. V., Spoelstra, J., Bickerton, G., Voralek, J., & Greer, C. W. (2013). Bacterial community evidence for anaerobic degradation of petroleum hydrocarbons in cold climate groundwater. *Cold Regions Science and Technology, 86*, 55–68.

Zhang, Z., Hou, Z., Yang, C., Ma, C., Tao, F., & Xu, P. (2011). Degradation of n-alkanes and polycyclic aromatic hydrocarbons in petroleum by a newly isolated *Pseudomonas aeruginosa* DQ8. *Bioresource Technology, 102*(5), 4111–4116.

Zhang, X., Xu, D., Zhu, C., Lundaa, T., & Scherr, K. E. (2012). Isolation and identification of biosurfactant producing and crude oil degrading *Pseudomonas aeruginosa* strains. *Chemical Engineering Journal, 209*, 138–146.

Zouboulis, A. I., & Moussas, P. A. (2011). Groundwater and soil pollution: bioremediation. In J. Nriagu (Ed.), *Encyclopedia of Environmental Health* (pp. 1037–1044). London: Elsevier.