51 Microbial Communities in Hydrocarbon-Contaminated Temperate, Tropical, Alpine, and Polar Soils

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Abstract: Microbial community structures in hydrocarbon-contaminated soils from different geographical origins, soil types and with different physico-chemical properties demonstrate important patterns that alter in response to the contamination event itself, and during subsequent biodegradation. The initial microbial community structure in a soil is a function of the geographical location, the properties of the soil and the environmental conditions. The addition of nutrients, levels of contamination, moisture and other physico-chemical parameters influence the predominant microbial populations. Arid, alpine and polar soils, which are typically nutrient- and moisture-limited are often dominated by K-strategists such as *Actinobacteria*, that are well adapted to low resource environments, and do not show major alterations as a result of hydrocarbon contamination. In hydrocarbon-contaminated soil, including alpine and polar soils, there is a dominance of *Proteobacteria* especially in the early stages of hydrocarbon degradation. All groups of *Proteobacteria*, with the possible exception of the *Epsilonproteobacteria*, respond positively to the influx of hydrocarbon substrates and also to situations in which supplemental nutrients (N, P) are supplied. This pattern is altered over time as hydrocarbon degradation reaches its plateau, and is frequently dominated by *Gammaproteobacteria*. In temperate and tropical soils, the microbial community structure is more diverse and the responses are not as pronounced, although again, the *Proteobacteria* are typically dominant following hydrocarbon contamination. As more information becomes available on the microbial community structures and their response at the community level, more effective biodegradation strategies can be developed to capitalize on this succession of active hydrocarbon degrading microorganisms.

1 Introduction

Human activities have generated a plethora of environmental pollutants, from heavy metals and radioactive materials to a wide range of organic compounds. One of the largest sources of environmental pollutants results from the use of fossil fuels to generate energy. Fossil fuels, in the form of coal, natural gas and petroleum, are used for heating, electrical power generation, and transportation of all kinds. They represent by far the largest source of energy for human activities, accounting for 86% of all energy consumption worldwide in 2003 (Energy Information Administration, 2005). Accordingly, the storage, transportation and combustion of fossil fuels release massive amounts of contaminating hydrocarbons. Virtually no environment on Earth is unaffected by hydrocarbon contamination: soils and sediments, groundwater, freshwater and oceans are all subjected to various degrees of contamination.

Ultra-high-resolution mass spectrophotometry recently indicated that there are more than 17,000 different compounds in crude oil (Marshall and Rogers, 2003). The compounds in this complex mixture have a range of physico-chemical properties that influence their biodegradability and ultimate environmental fate. Representatives from all three major domains of life have demonstrated the ability to metabolize hydrocarbons to various degrees and there are currently in excess of 200 bacterial, algal and fungal genera that are known to use hydrocarbons as carbon and energy sources (Head et al., 2006; Prince et al., 2003).

The extent to which microorganisms participate in the biodegradation of hydrocarbons appears to be a function of the ecosystem and the local environmental conditions (as reviewed by Leahy and Colwell, 1990). Prior exposure of a microbial community to anthropogenic and/or natural sources of hydrocarbons is an important factor in determining the rate of biodegradation.

The objectives of this Chapter are to survey the impact of hydrocarbon contamination on microbial community dynamics in various soil ecosystems and summarize changes in the microbial community structure and composition associated with contamination events, bioremediation treatments of contaminated sites or natural attenuation. *Table 1* provides a summary of key studies that have shown shifts in the microbial community structure associated with hydrocarbon contamination. Monitoring the hydrocarbon impacts on soil microbial communities will help identify common patterns associated with soil hydrocarbon biodegradation and consequently lead to the development of better tools for rapidly and reliably determining the effectiveness of bioremediation treatments on biodegradation processes.

2 Influence of Physico-Chemical Parameters

Hydrocarbon biodegradation can occur in a variety of environments, some of which are considered extreme (Margesin and Schinner, 2001), and include such important factors as soil type, contamination type and concentration, temperature, pH, salinity, nutrient status, etc. These parameters also influence the density and composition of the indigenous microbial community, the activity of which will dictate the rate of hydrocarbon degradation.

An examination of the microbial community profiles of three different soil types contaminated with diesel fuel showed that there was no community convergence and that the profiles that developed were dependent on soil type (Bundy et al., 2002). Hamamura et al. (2006) extended this concept and examined seven diverse soil types from six geographically distinct sites in the USA. Hydrocarbon degradation activity and microbial populations differed between the soil types, but the isolated phylotypes were similar to other hydrocarbon degraders (*Nocardioides*, *Collimonas* and *Rhodococcus*) from the same systems. One of the important conclusions from this study was that soil type was an important factor in determining the microbial community structure in the soil.

Contaminant concentration and type also have a profound influence on the structure of the developing microbial communities. For example, Muckian et al. (2007) noted a correlation between polycyclic aromatic hydrocarbon structure (number of rings) and community composition in soil from a timber treatment facility in southern Ireland and Hawle-Ambrosch et al. (2007) demonstrated a preferential degradation of shorter-chain alkanes, which was more efficient with microbial consortia. Recently, Powell et al. (2006) used a quantitative-PCR method targeting the *alkB* gene, encoding alkane hydroxylase, to show that the proportion of *alkB* genotypes was positively correlated to the concentration of alkanes in a contaminated Antarctic soil.

Nutrient treatments also have an impact on bioremediation effectiveness and on the resulting microbial community structure. Denaturing gradient gel electrophoresis (DGGE) was used to show dramatic shifts in microbial community structure during bioremediation of creosote contaminated soil in Spain (Viñas et al., 2005). The early stages of bioremediation were dominated by *Alphaproteobacteria* (*Sphingomonas* and *Azospirillum*) in all treatments but in the later stages the *Gammaproteobacteria* (*Xanthomonas*), *Sphingomonas* and the *Cytophaga/Flavobacterium/Bacteriodes* (CFB) group were dominant in non-nutrient treated systems, while *Xanthomonas* and *Betaproteobacteria* (*Alcaligenes*, *Achromobacter*) and *Sphingomonas* dominated in the nutrient treatments. Baek et al. (2007) examined a variety of bioremediation scenarios on crude oil contaminated soils including natural attenuation,

Table 1

Key studies describing microbial population shifts (or differences) of pristine and hydrocarbon-contaminated soils

Habitat and/or Geographic location	Soil type	Anthropogenic contamination	Treatment specifics	Technique utilized to describe communities	Pristine/Initial soil microbial community (dominant members)	Contaminated/Final soil microbial community (dominant members)	Dominant/common microbial genera of contaminated samples (taxonomic grouping)	Reference
Temperate								
6 US sites (AZ, OR, IN, VA, O, MT)	Various	Crude-oil	NA	DGGE band sequences		Diverse communities amongst sites	*Rhodococcus (Actinobacteria)*	Hamamura et al. (2006)
						Actinobacteria		
						Betaproteobacteria		
						Gammaproteobacteria		
Wood treatment plant, Barcelona, Spain	Loamy clay soil	Creosote	Nutrient	DGGE band sequences	*Alphaproteobacteria*	*Non-nutrient amended*	*Sphingomonas (Alphaproteobacteria)*	Viñas et al. (2005)
						Alphaproteobacteria		
						Gammaproteobacteria	*Xanthomonas (Gammaproteobacteria)*	
						CFB		
						Nutrient amended		
						Alphaproteobacteria	*Achromobacter (Betaproteobacteria)*	
						Betaproteobacteria		
						Gammaproteobacteria	*Alcaligenes (Betaproteobacteria)*	
							Sphingomonas (Alphaproteobacteria)	
							Xanthomonas (Gammaproteobacteria)	

Oil refinery, Shizuoka, Japan	Fine sand with silt	Crude oil	NA	16S rRNA gene library	*Alphaproteobacteria* (2%)	*Alphaproteobacteria* (2%)	*Alkanindiges (Gammaproteobacteria)*	Kasai et al. (2005)
					Betaproteobacteria (72%)	*Betaproteobacteria* (54%)		
					Deltaproteobacteria (2%)	*Deltaproteobacteria* (6%)		
					Gammaproteobacteria (4%)	*Gammaproteobacteria* (16%)		
					Epsilonproteobacteria (ND)	*Epsilonproteobacteria* (6%)		
					Bacteroidetes (12%)	*Bacteroidetes* (2%)		
					Acidobacteria (8%)	*Acidobacteria* (4%)	*Sterolibacterium (Betaproteobacteria)*	
					Actinobacteria (ND)	*Actinobacteria* (2%)	*Sulfuricurvum (Epsilonproteobacteria)*	
					Archaea	*Archaea*	*Variovorax (Betapoteobacteria)*	
					Methanosarcinales (ND)	*Methanosarcinales* (15%)	*Archaea*	
					Crenarchaeota (26%)	*Crenarchaeota* (41%)	*Methanosaeta (Euryarcheota)*	
Guadalupe oil field, CA, USA	Highly weathered soil	Petroleum hydrocarbons	Nutrient	T-RFLP		*Dominant peaks (rapid degraders)*	*Flavobacterium* (CFB)	Kaplan and Kitts (2004)
						Flavobacterium	*Pseudomonas (Gammaproteobacteria)*	
						Pseudomonas		

Table 1 (Continued)

Habitat and/or Geographic location	Soil type	Anthropogenic contamination	Treatment specifics	Technique utilized to describe communities	Pristine/Initial soil microbial community (dominant members)	Contaminated/Final soil microbial community (dominant members)	Dominant/common microbial genera of contaminated samples (taxonomic grouping)	Reference
Capitol Heights, MD, USA	Highly weathered soil	Petroleum hydrocarbons	31 days time point	16S rRNA gene library	*Actinomycetales* (4%)	*Actinomycetales* (15%)	*Pseudomonas (Gammaproteobacteria)*	Mills et al. (2003)
					Bacillus/Clostridium (11%)	*Bacillus/Clostridium* (ND)		
					Bacillus/Lactobacillus (ND)	*Bacillus/Lactobacillus* (19%)	*Sphingomonas (Alphaproteobacteria)*	
					CFB (7%)	CFB (ND)		
					Alphaproteobacteria (19%)	*Alphaproteobacteria* (15%)		
					Betaproteobacteria (37%)	*Betaproteobacteria* (7%)	*Stenotrophomonas (Gammaproteobacteria)*	
					Gammaproteobacteria (7%)	*Gammaproteobacteria* (41%)		
					Deltaproteobacteria (15%)	*Deltaproteobacteria* (4%)		
Dover, Ohio, USA	Loam soil	Phenanthrene and hexadecane	Surfactant	DGGE band sequences		*Hydrocarbon only*	*Alcaligenes (Betaproteobacteria)*	Colores et al. (2000)
							Nocardia (Actinobacteria)	
						Rhodococcus	*Pseudomonas (Gammaproteobacteria)*	
						Nocardia		
						Hydrocarbon & high surfactant concentration		
						Pseudomonas		
						Alcaligenes	*Rhodococcus (Actinobacteria)*	

Rositz, Germany	Silty loam	Alkanes, PAHs, BTEX, phenol	NA	16S rRNA gene library	Dominant:		*Pseudomonas (Gammaproteobacteria)*	Popp et al. (2006)
					Gammaproteobacteria		*Acinetobacteria (Betaproteobacteria)*	
					Alphaproteobacteria			
					Betaproteobacteria			
					Others:			
					Actinobacteria			
					Firmicutes			
					Betaproteobacteria		*Sphingomonas (Alphaproteobacteria)*	
					Bacteriodetes		*Acidovorax (Betaproteobacteria)*	
					Epsilonproteobacteria		*Thiobacillus (Betaproteobacteria)*	
Fowler beach, DE, USA	Sand	Crude oil	Nutrients & inocula	DGGE band sequences	*Alphaproteobacteria* (ND)	*Alphaproteobacteria* CFB	*Sphingomonas (Alphaproteobacteria)*	Macnaughton et al. (1999)
Arid								
Patagonia, Argentina	Semi-arid soil	Crude oil	NA	Culturing		Gram-negative genera	*Pseudomonas (Gammaproteobacteria)*	Pucci et al. (2000)
					Gram-positive genera	Fewer Gram-positive genera		
Tropical								
Bangkok, Thailand	Sand-textured soil	Lubricant oil	N and P addition	DGGE band sequences		*Unique DGGE bands*	*Bacillus (Firmicutes)*	Supaphol et al. (2006)
						Bacillus		
						Microbacterium	*Microbacterium (Actinobacteria)*	
						Pseudomonas	*Pseudomonas (Gammaproteobacteria)*	

Table 1 (Continued)

Habitat and/or Geographic location	Soil type	Anthropogenic contamination	Treatment specifics	Technique utilized to describe communities	Pristine/Initial soil microbial community (dominant members)	Contaminated/Final soil microbial community (dominant members)	Dominant/common microbial genera of contaminated samples (taxonomic grouping)	Reference
Polar								
Ross Is., Antarctica	Stony, gravel sands (typic anhyorthel)	Hydrocarbons	NA	16S rRNA gene library	*Fibrobacter/ Acidobacterium* (20%)	*Fibrobacter/ Acidobacterium* (ND)	*Arthrobacter (Actinobacteria)*	Saul et al. (2005)
					Actinobacteria (17%)	*Actinobacteria* (6%)		
					CFB (10%)	CFB (ND)	*Brevundimonas (Alphaproteobacteria)*	
					Alphaproteobacteria (3%)	*Alphaproteobacteria* (33%)	*Pseudomonas (Gammaproteobacteria)*	
					Betaproteobacteria (4%)	*Betaproteobacteria* (14%)	*Sphingomonas (Alphaproteobacteria)*	
					Gammaproteobacteria (1%)	*Gammaproteobacteria* (29%)	*Xanthomonas (Gammaproteobacteria)*	
Various soils, Ross Sea region, Antarctica	Various	Mixed oils	NA	Culturing	*Fungi*	*Fungi*		Aislabie et al. (2001)
					Chrysosporium			
					Geotrichum	*Phialophora*		
Alpine								
Tyrol, Austria	Moraine and alluvial gravel, gneiss soil	Diesel oil	NA	DGGE band sequences	*Actinobacteria* (19%)	*Actinobacteria* (20%)	*Aquimonas (Gammaproteobacteria)*	Labbé et al. (2007)
					Alphaproteobacteria (46%)	*Alphaproteobacteria* (24%)	*Arthrobacter (Actinobacteria)*	
					Betaproteobacteria (ND)	*Betaproteobacteria* (24%)	*Brevundimonas (Alphaproteobacteria)*	
					Gammaproteobacteria (ND)	*Gammaproteobacteria* (8%)	*Sphingomonas (Alphaproteobacteria)*	

NA Not applicable; *ND* Not detected; *CFB Cytophaga-Flexibacter-Bacteroidetes*

biostimulation, biosurfactant addition, bioaugmentation and combined treatments. Although the amount of hydrocarbon remaining after 120 days was similar for all treatments, the DGGE profiles showed considerable differences before, after, and between all treatments.

The limited information available on hydrocarbon degradation in saline environments indicates that at high salt concentrations (20%) hydrocarbon degradation is severely inhibited (Kleinsteuber et al., 2006; Riis et al., 2003; Ward and Brock, 1978). Microbial population shifts were observed during increasing salt concentrations, whereby *Sphingomonas* dominated at low salt concentrations, but higher salt concentrations selected for *Ralstonia*, *Halomonas*, *Dietzia* and *Alcanivorax* spp. in one study (Riis et al., 2003) and *Cellulomonas*, *Bacillus, Dietzia* and *Halomonas* in another study (Kleinsteuber et al., 2006).

There is also evidence that polycyclic aromatic hydrocarbons are biodegraded under acidic conditions (pH 2); however, no individual bacterial isolates could be identified that degraded the tested substrates, and genes that are known to be involved in the degradation of these compounds were not detected (Stapleton et al., 1998). The degradation was carried out by a microbial consortium composed of yeast, fungi and bacteria.

3 Temperate Soils

It is generally well established that microbial community structures alter in response to perturbation by contamination. Greene et al. (2000) showed a succession of microbial communities in three aromatic hydrocarbon contaminated soils in Alberta, Canada, from *Pseudomonas* to *Rhodococcus* to *Alcaligenes*. Colores et al. (2000) used radiolabeled hexadecane and phenanthrene in soil microcosms to determine the effect of surfactant on degradation. When surfactant was added at a concentration above the critical micelle concentration, degradation was inhibited. DGGE analysis showed that the presence of hydrocarbon stimulated *Rhodococcus* and *Nocardia* (dominant DGGE bands, not detected in control soils), which were subsequently displaced by *Pseudomonas* and *Alcaligenes* at high surfactant concentrations. Culturing of these isolates demonstrated that they were in fact using the tested surfactant as a carbon source.

A study of bacterial succession in a petroleum land treatment unit in a Guadalupe oil field on the central Californian coast revealed different terminal-restriction fragment length polymorphism (T-RFLP) peaks at different stages of degradation (Kaplan and Kitts, 2004). When the rate of degradation was at its highest, *Flavobacterium* and *Pseudomonas* dominated, which subsequently returned to basal levels as hydrocarbon degradation rates began to decrease. At this time, *Thermomonas*, *Azoarcus*, an unknown and *Rhodanobacter* became the predominant phylotypes. There was a strong correlation between the bacterial succession of dominant phylotypes and hydrocarbon biodegradation.

Using several molecular profiling methods, Mills et al. (2003) demonstrated a shift in the microbial community structure following hydrocarbon contamination, the major changes associated with a decrease in *Betaproteobacteria* and an increase in *Gammaproteobacteria* after 21 days. The latter group decreased to unamended levels after 31 days of treatment. Community structural differences were also observed by Kasai et al. (2005), who found a different community in a petroleum contaminated zone compared to an adjacent uncontaminated site in Japan. A comparison of clone libraries from the two soils demonstrated that *Epsilon-* and *Gammaproteobacteria*, *Crenarchaeota* and *Methanosarcinales* were only detected at significant levels in the contaminated soil. Popp et al. (2006) showed that

hydrocarbon-contaminated soil in Germany was dominated by *Gammaproteobacteria* (especially *Pseudomonas* spp.), *Alphaproteobacteria* and *Betaproteobacteria*, with the presence of *Actinobacteria*, *Firmicutes*, *Bacteroidetes* and *Epsilonproteobacteria* detected in lower numbers.

Kasai et al. (2001) investigated microbial communities in paste samples of heavy oil from the Nakhodka tanker oil-spill on the beaches of Japan and the associated seawater. Analysis of the community profiles using DGGE revealed differences between the contaminated seawater and the beaches, demonstrating a dominance of *Alphaproteobacteria* in seawater, however, the beach samples, showed a dominance of *Hydrogenophilus thermoluteolus*. This bacterium is not known to degrade hydrocarbons, although known hydrocarbon degraders such as *Sphingomonas*, *Alcanivorax borkumensis* and *Stenotrophomonas* were also detected in the beach samples.

Quatrini et al. (2008) used a culture-based approach to investigate the petroleum hydrocarbon degrading potential of indigenous microbes in a sandy Mediterranean coastal environment that was contaminated with petroleum hydrocarbons. The microorganisms isolated using fuel mix as sole carbon and energy sources were mainly Gram-positive rods related to *Nocardia, Rhodococcus and Gordonia*, with a single Gram-negative isolate identified as *Pseudomonas*. The study concluded that *Actinomycetes* might have a major role in the bioremediation of alkane contaminated, dry, resource limited soils such as beaches and shorelines. Pucci et al. (2000) used a similar culture-centric approach to investigate the influence of crude oil contamination on bacterial communities of semiarid soils in Patagonia. Initially, there was a microbial population dominated by Gram-positive *Actinomycetales* (*Dietzia*, *Gordonia*, *Nocardia*, *Rhodococcus*, *Streptomyces*) but following exposure to crude oil, Gram-negative bacteria such as *Pseudomonas*, became predominant, accompanied by lower proportions of *Actinomycetales* (*Corynebacterium, Nocardia, Rhodococcus, Streptomyces, Gordonia*). Macnaughton et al. (1999) investigated community changes on an artificially contaminated coastal region of Delaware using culture-independent techniques (DGGE and phospholipid fatty acid analysis (PFLA)). PFLA results showed a shift in the community from eukaryotic biomass to Gram-negative bacteria during oil exposure. The DGGE analysis showed considerable microbial community changes between treatments, again with a dominance of Gram-negative bacteria in the *Alphaproteobacteria* and CFB phylum following oil treatment, while *Alphaproteobacteria* were never detected in un-oiled controls.

Other studies have also shown changes in the microbial community structures associated with many factors that include the type and concentration of contamination, nutrient status, soil type and other physico-chemical properties, in addition to changes associated with season and geographical location. Taken together, these results clearly demonstrate the dynamic nature of indigenous soil microbial populations, and emphasize that each ecosystem needs to be evaluated separately. Nevertheless, common patterns in microbial community structures and how they adjust to these factors are emerging.

4 Tropical Soils

Tropical soils contain a plethora of potential hydrocarbon degrading microorganisms including bacteria, yeast and fungi. Chaillan et al. (2004) isolated aerobic hydrocarbon-degrading microorganisms from soils and freshwater ponds located in southeast Kalimantan (Borneo Island, Indonesia) contaminated for several years by Indonesian crude oil. Bacterial strains of the genera *Aeromicrobium*, *Brevibacterium*, *Burkholdia, Dietzia*, *Gordonia* and *Mycobacterium*, the fungi *Amorphoteca, Aspergillus*, *Fusarium*, *Graphium, Neosartorya, Paecilomyces*,

Penicillium, and *Talaromyces* and yeasts belonging to *Candida*, *Pichia* and *Yarrowia* were all identified. Supaphol et al. (2006) investigated the effect of the addition of N and P on the bioremediation of oil contaminated tropical soil collected from around an oil storage tank in Bangkok, Thailand. DGGE was used to compare the community structure over time and predominant bands were identified as *Bacillus*, *Microbacterium* and *Pseudomonas*. These three genera were isolated into pure culture using lubricating oil as a sole carbon source and accounted for 38% of the 317 isolates obtained: 77 isolates were *Bacillus*, 35 *Microbacterium* and 9 *Pseudomonas*. These isolates were much more efficient at degrading the lubricating oil in liquid media or in sand as consortia rather than individually.

A recent review of the microbiology of oil-contaminated desert soils in Kuwait indicated that nutrient starvation is the most severe limitation (Radwan, 2008). Despite the large temperature difference, desert soils share important features with polar and alpine soils in that they are nutrient-poor and have low available water. In the Kuwaiti desert, the predominant indigenous oil degrading bacteria were *Micrococcus*, *Pseudomonas*, *Bacillus*, *Arthrobacter*, *Rhodococcus* and *Streptomyces*. Oil utilizing fungi, including *Aspergillus*, *Penicillium*, *Fusarium* and *Mucor*, were also identified. A significant population density of thermophilic hydrocarbon degrading bacteria related to *Geobacillus stearothermophilus* were also detected in desert soil. These microorganisms were consistently found in higher numbers in oil contaminated soils than in pristine soils. In the presence of plants, oil degrading rhizosphere bacteria, the most prevalent being *Cellulomonas, Rhodococcus* and *Arthrobacter,* were enriched.

5 Alpine Soils

Alpine microorganisms with the ability to degrade hydrocarbons at low temperature have been comprehensively reviewed by Margesin (2007). Earlier studies demonstrated that pristine alpine soils do contain high numbers ($\sim 10^3$ to 10^4 cells g^{-1} soil) of indigenous bacteria able to utilize diesel oil as a sole carbon source (Margesin and Schinner, 1997) which was later corroborated by Margesin et al. (2003) showing that both contaminated and uncontaminated alpine soils contain significant oil degrading bacterial populations ($\sim 10^5$ to 10^7 cells g^{-1} soil). What is interesting for the recent study is that cold-adapted degraders (10°C) were 2–3 orders of magnitude higher than their mesophilic (37°C) counterparts, emphasizing the importance of cold–adapted microbes in the bioremediation of hydrocarbons (Margesin et al., 2003). Margesin et al. (2003) also found that catabolic genotypes involved in the degradation of n-alkanes from targeted Gram-negative bacteria (*Pseudomonas putida* and *Acinetobacter*) were significantly higher in contaminated (50–75%) than the corresponding pristine soils (0–12.5%) indicating an enrichment effect for microorganisms capable of hydrocarbon degradation. Furthermore, there was a higher significant positive correlation between the level of contamination and the number of genotypes of Gram-negative (*P. putida* and *Acinetobacter*) rather than Gram-positive genotypes (*Rhodococcus* and *Mycobacterium*).

A similar shift in the microbial community structure, with respect to an enrichment of Gram-negative *Proteobacteria*, was recently demonstrated in a phylogenetic survey of pristine and hydrocarbon contaminated alpine soils (Labbé et al., 2007). DGGE analysis demonstrated a predominance of *Actinobacteria* and *Proteobacteria* in pristine (18 and 73%, respectively) and contaminated soils (20 and 76%, respectively). However, the classes of *Proteobacteria* were directly related to the presence (or absence) of contamination. In particular, the proportion of *Alphaproteobacteria* was greater in pristine soils (46%) than in contaminated soils (24%) and

Beta- (8%) and *Gammaproteobacteria* (24%) were only detected in contaminated soils. Moreover, of all detected classes of bacteria, only the *Gammaprotebacteria* were significantly ($P < 0.01$) positively correlated to that of soil TPH content.

6 Polar Soils

Various studies have shown that there are large numbers of indigenous microbes in polar soils that are capable of degrading hydrocarbons, with culturable population densities as high as 10^5 cells g^{-1} soil (Braddock et al., 1997; Coulon et al., 2005; Saul et al., 2005; Whyte et al., 2001).

Cold-adapted hydrocarbon degraders have been isolated without difficulty from many polar soils, and the bioremediation of hydrocarbon-contaminated polar soils has been recently reviewed (Aislabie et al., 2006). Common examples of hydrocarbon-degrading bacteria include psychrotolerant *Pseudomonas*, *Sphingomonas* and *Rhodococcus* strains isolated from Arctic environments (Thomassin-Lacroix et al., 2002; Whyte et al., 1997, 1998) and Antarctic soils (Aislabie et al., 2000; Saul et al., 2005). Deppe et al. (2005) used DGGE to show that nine species closely related to the genera *Pseudoalteromonas*, *Pseudomonas*, *Shewanella*, *Marinobacter*, *Psychrobacter* (*Gammaproteobacteria*) and *Agreia* were present in an Arctic consortium degrading crude oil at 4°C. Interestingly, in different combinations, the isolated strains could not significantly degrade crude oil, indicating the importance of this mixed community. These same bacterial groups were present in hydrocarbon contaminated alpine soils (Margesin et al., 2003). Thomassin-Lacroix et al. (2002) found *Pseudomonas*, *Rhodococcus* and *Sphingomonas* sp. were enriched from Arctic soils contaminated with diesel oil, and Eriksson et al. (2001) has shown that *Rhodococcus* is adept at surviving laboratory-simulated freeze-thaw cycles (7°C and −5°C), subsequently becoming a predominate member of the microbial community degrading hydrocarbons within an Arctic tundra soil sample.

At present, there are few molecular-based studies in the literature comprehensively describing the microbial communities of polar soils affected by hydrocarbon degradation. A study by Juck et al. (2000) presents one of the first studies to compare community structures between pristine and petroleum hydrocarbon contaminated soils in the Canadian Arctic using DGGE analysis. The predominant band patterns (63.6%) represented high G + C microorganisms in the *Actinomycetales* order and 36.4% belonged to the *Proteobacteria*, with the *Gammaproteobacteria*, comprised primarily of the genera *Xanthomonas*, *Halomonas* and *Methylobacter*, being the most significant members (62.5%) of the *Proteobacteria*. The results of this study indicated that geographic origin was a more important determinant in clustering of DGGE profiles than petroleum contamination.

Studies comparing and contrasting microbial communities between contaminated and non-contaminated polar soils have only been presented by two key studies (Aislabie et al., 2001; Saul et al., 2005) both situated in the Antarctic. Aislabie et al. (2001) used a culture-based approach to compare isolated microorganisms from pristine and contaminated Antarctic soils. A shift in microbial inhabitants was observed: yeast was only cultivated from oil contaminated soils and higher numbers of filamentous fungi were isolated from contaminated sites compared to pristine soils. The filamentous fungi shifted from *Chrysosporium* dominance in control soils to *Phialophora* dominance in hydrocarbon contaminated soils. Saul et al. (2005) present the first comprehensive comparison of microbial communities between pristine and hydrocarbon impacted soils around Scott Base, Antarctica, using a culture-based and culture-independent approach. Members of the *Actinobacteria* were found in both soil types,

but distinct differences were detected between contaminated and pristine soils. For example, bacteria belonging to the *Fibrobacter/Acidobacterium, CFB, Deinococcus/Thermus* and low G + C Gram-positive genera were almost exclusively present in pristine soils, whereas contaminated soils were dominated by species related to the *Proteobacteria*, including *Pseudomonas*, *Sphingomonas* and *Variovorax*.

There is virtually no information available on the effects of hydrocarbons on permafrost microbial communities although the structures of several of these communities have been reported (Gilichinsky et al., 2007; Steven et al., 2007; Vishnivetskaya et al., 2006). In one study, the biodegradation of hydrocarbons in permafrost incubated at 5°C was observed, but the characteristics of the microorganisms and microbial community were not examined (Børresen et al., 2003).

7 Research Needs

Over the last decade or so, there have been numerous developments in methods to analyze complex microbial communities and their functions. The exploitation of new proteomics and genomics tools (Zhao and Poh, 2008) to establish relationships between microbial community structure and function will help provide much needed insight into the degradation of environmental pollutants, how microorganisms respond to different stimuli, and especially how degradation systems function at the community level. A recent review of molecular techniques to characterize microbial communities in contaminated soil and water has presented methods, including high throughput techniques, to link microbial phylogeny and ecological function (Malik et al., 2008). Stable isotope probing (SIP) is another technique that can distinguish between who is there and who is actually functioning in the system under evaluation by coupling stable isotopic compounds with molecular techniques. As recently reviewed by Madsen (2006), important information is presented about how to use SIP and how to interpret subsequent results. The key is to develop data on both the phylogenetically dominant and active hydrocarbon-degrading strains in oil contaminated environments.

Due to the metagenomics revolution, genomics-based databases that are growing at immense rates, and will enable more complex communities to be assembled to give a more meaningful understanding of the phylogeny/function relationships. Large managed databases of microbial diversity, and changes associated with both contamination events and bioremediation are required; however, it is essential to develop a uniform way of presenting data since, at present, there is a considerable amount of disparate data that defies comparison. Therefore, there is a clear need to develop standardized analytical approaches and tools to enable the development of comprehensive databases that would allow researchers and practitioners to access and compare data from sites having different contaminant types, geographical locations, nutritional and physico-chemical properties. A recent article by Marzorati et al. (2008) presents a means to normalize DGGE data so it can be compared between laboratories and environments. This could be a major step forward in the use of screening data (as an alternative to large scale sequencing) to evaluate different environments.

In addition to all the available direct molecular approaches, it is still important to isolate and characterize pure cultures of hydrocarbon-degrading microorganisms that inhabit less-commonly studied environments. The development of specialized culturing techniques, suitable to, and representative of the many unexplored environments that may contain unique microorganisms, is another area that requires additional research effort.

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