Microbial diversity in oiled and un-oiled shoreline sediments in the Norwegian Arctic

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ABSTRACT

Field trails were performed in the summer of 1997 on the island of Spitzbergen (approximately 78°N, 17'E). Approximately 5 L m^{-2} of an IF-30 intermediate fuel grade oil was applied to 140 meters of shoreline. The oiled shoreline was divided into four plots with one plot left untreated, and two plots tilled to a depth of approximately 20 cm. Four applications of fertilizer were applied over a two-month period to one of the tilled plots, and also to an untilled plot. The effect of bioremediation on the microbial community was evaluated using phospholipid fatty acid analysis, hydrocarbon degradation gene probes, and 16S rDNA gene based phylogenetic analysis. Clear differences were observed between fertilized and un-fertilized beaches indicating that bioremediation stimulated the hydrocarbon degrading community in an Arctic environment.

Introduction

Bioremediation has proven to be an environmentally acceptable and cost-effective treatment of oiled shorelines in temperate climates [16, 20, 4, 13, 27, 18], and there is good reason to believe that similar results can be expected in the Arctic [23, 25, 26]. Hydrocarbons are generally quite biodegradable and oil-degrading microbes are ubiquitous [17], but oil biodegradation is limited in most marine environments by sub-optimal levels of nutrients such as biologically available nitrogen and phosphorus. Bioremediation of oil spills has thus focused on adding fertilizers to oiled shorelines to alleviate this limitation. Bioremediation of oiled marine shorelines has been shown to increase the number of hydrocarbon degrading microorganisms, their hydrocarbon mineralization potentials and the rate of hydrocarbon degradation [14, 19, 4, 18]. However, the effects on the microbial community structure associated with oiling and bioremediation have not been well characterized. In this work we evaluate the effect of nutrient addition on the metabolic status, potential for aromatic hydrocarbon degradation, and the phylogenetic diversity of the microbial community in oiled Arctic shoreline sediments.

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Methods and Materials

Beach Treatment and Sample Collection

The experiment took place on shorelines near Sveagruva, Spitzbergen (approximately 78°N, 17'E) in the summer of 1997. Air and water temperatures in August, when oil and initial treatments were applied, were 3-7 °C, although interstitial water in the shoreline was typically slightly warmer (4-9 °C). The site where bioremediation was used was a relatively sheltered gravel beach above a shallow subtidal environment. The beach faced approximately southeast, and had an approximately 10-km fetch to the Paulabreen glacier. Winds from the southeast generated 10-30 cm waves, and on occasion deposited small icebergs near the test beaches. Winds from other directions left the beach rather sheltered.

The oil used in this study was an IF-30 intermediate fuel grade made by mixing relatively heavy distillate with lighter fractions to obtain the desired viscosity. Oil was applied at approximately 5 L m^{-2} directly onto the shoreline during the afternoon low tide of 29 July 29 1997. It penetrated to a depth of approximately 15 cm. A total of 140 meters of shoreline was oiled, and this was subsequently divided into four plots, as shown in Fig. 1. These plots were treated on the morning low tide of 6 August 1997, approximately one week after the oil was applied. One plot was left untreated, and two plots were tilled to a depth of approximately 20 cm by drawing tines through the plot both down and across the beach (once in each direction). This tilling extended to just beyond the depth of oil penetration. Fertilizer was applied (see Table 1) to one of these tilled plots after the first tilling, and also to an untilled plot. Subsequent applications of fertilizer to these two plots are described in Table 1.

Table 1. Bioremediation treatment.

Date	**Fertilizer**
6 August 1997, 7 days after oiling	100 g m^{-2} prilled ammonium nitrate (Hydro, Sweden); 10 g m^{-2} superphosphate (Hydro, Sweden); 1 g m^{-2} ferrous sulfate (89% $FeSO_4.H_2O$ 5% $MgSO_4$, 0.2% $MnSO_4$, Christen Hoeg A/S, Norway);0.1 g m^{-2} yeast extract (Sigma, USA)
13 August 1997, 14 days after oiling	140 g m^{-2} Inipol SP1 (CECA, Paris La Defense, France), a slow-release formulation containing 18% nitrogen as ammonium, and 1% phosphorus (as P_2O_5); 1 g m^{-2} ferrous sulfate and 0.1 g m^{-2} yeast extract.
29 August 1997, 30 days after oiling	100 g m^{-2} Inipol SP1
5 October 1997, 65 days after oiling	50 g m^{-2} prilled ammonium nitrate; 5 g m^{-2} superphosphate; 1 g m^{-2} ferrous sulfate; 0.1 g m^{-2} yeast extract; 70 g m^{-2} Inipol SP1.

Sampling

Interstitial water from the beaches was obtained from perforated plastic wells (approximately 30 cm deep and 4 cm in diameter) using a 60 ml syringe and an extension tube that extended to the bottom of the wells. Samples were collected from within the oiled zone of the sediment. Groups of three wells were placed at four locations on each test plot,

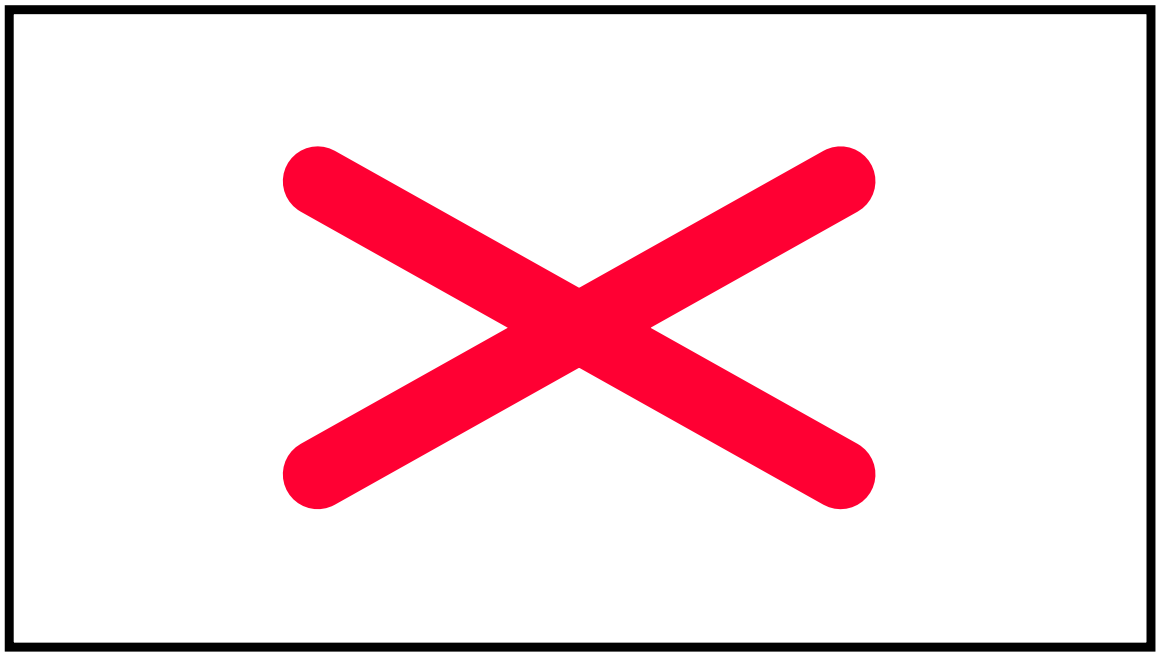

Fig. 1. Schematic of the shoreline used in the field trial. The top section shows the layout of the shoreline plots with the approximate positions of high water Spring and low water Spring tide levels. Neap tides usually covered the plots at high water. The bottom section shows the sampling areas within one of the test plots. Sediment samples were taken around each of the sampling wells, with care to ensure that samples were from undisturbed sediment. Interstitial water was removed from the sampling wells on falling tides when the wells were well clear of the water.

as shown in Fig. 1, and approximately 100 ml of interstitial water was collected at each location as the tide receded. Care was taken to ensure that no air bubbles were introduced during collection, and dissolved oxygen in the water samples was measured immediately, using Chemetrics K-7512 test kits (Chemetrics, Calverton, VA). Nitrate, ammonium and phosphate levels in the interstitial water samples were measured within one hour of sample collection using Chemetrics K6902, K-1510 and K-8510 kits. Laboratory studies confirmed that these tests accurately measured the nutrients down to a detection limit of approximately 1 μM phosphate, 6 μM ammonia and 2 μM nitrate.

Sediment samples for microbial community analysis were taken on 7 October 1997, 67 days after oiling and two days after the last fertilizer addition. Samples were stored frozen prior to analysis. Four samples were taken around each well, giving a total of twelve samples per oiled beach segment. Analysis was performed on composites of these twelve samples. A composite sediment sample from an un-oiled section of the beach approximately 300 meters from the test site was used as a remote beach un-oiled Control.

Phospholipid Fatty Acid Analysis

Phospholipid fatty acid analysis was performed by Microbial Insights (Rockford, TN) using their standard protocols [30, 12].

Catechol 2,3 Dioxygenase Analysis

PCR amplification of catechol 2,3 dioxygenase (C23O) genes was performed by Microbial Insights (Rockford, TN) using their standard protocols [12]. DNA extracted from sediment samples was amplified using the primer pair, 5'-TGG CCG CGC GAT CTG AAA GGT ATG G-3' and 5'-GAT ATC GAT MGA KGT GTC GGT CAT G-3' (M= A, C, K = G,T), which targets the C23O genes encoded by the *nahH* on plasmid NAH7 of *Pseudomonas putida* and the *xylE* gene of the TOL plasmid WWO [3].

DNA Extraction and Purification for 16S Analysis

A modified direct lysis method was used to extract DNA from the sediment [1, 10]. Eight grams of sediment was suspended in 20 ml of wash buffer containing 0.3% (w/v) sodium pyrophosphate and 3% (w/v) polyvinylpyrolidone (PVP) and mixed by shaking at 150 rpm for 15 min at room temperature. The sediment was pelleted by centrifugation at 27,000 x g for 20 min. The supernatant was decanted and the sediment washed once more. The sediment was resuspended in 20 ml of bacterial lysis buffer (10 mM Tris-HCl, 200 mM Na_2EDTA, 0.3 M sucrose, 2% (w/v) PVP (pH 8.0), and 5 mg/ml lysozyme). After incubation at room temperature for one hour with gentle shaking, 2.0 ml of proteinase K solution (20 mg/ml in 50 mM Tris-HCl, 25 mM Na_2EDTA, pH 8.0) and 2.0 ml of 20% (w/v) sodium dodecylsulfate solution were added. After incubation for 30 min at room temperature with gentle shaking, 2 ml of 5 M NaCl and 2 ml of 10% (w/v) hexadecyltrimethylammonium bromide in 0.7 M NaCl solution were added and the sediment suspension incubated for one hour at 65°C. The suspension was centrifuged at 8,000 x g for 10 min at room temperature to remove sediment. The supernatant was extracted with an equal volume of phenol:chloroform:isoamyl alcohol solution (25:24:1). DNA was precipitated by adding 0.6 volume of isopropanol and incubating at -20 °C overnight. DNA was collected by centrifugation at 27,000 x g for 30 min, washed with 70% ethanol, and reconstituted in sterile TE buffer (10 mM Tris-HCl, 1 mM Na_2EDTA, pH 8.0).

PCR Amplification and Cloning of 16S Genes

Ribosomal RNA genes (rDNA) were amplified by the polymerase chain reaction (PCR), using the universal 16S ribosomal RNA (rRNA) primer 1392R (5'-ACG GGC GGT GTG TRC -3') and the bacterial primer 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') (numbers of the primers correspond to *Escherichia coli* positions and R = purine, M= A,C). PCR was performed in 100 µl reaction volumes, using 100 ng of template DNA, 2.5 units of AmpliTaq DNA polymerase (Perkin Elmer Cetus, Norwalk, CT), 20 pmoles of each primer, 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 2.5 mM $MgCl_2$ and 10 µM of each dNTP. Target rDNA was amplified using a PCR protocol of denaturing at 92 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 1 min for 30 cycles with a final extension at 72 °C for 15 min. Amplified DNA was further purified using Amicron microconcentrators following manufactures instructions (Amicron, Beverly, MA). For each sample, 5 PCR reactions were performed and the products pooled. Amplification of the target rDNA was confirmed by agarose gel electrophoresis.

The PCR fragment was cloned using the Promega pGem-T cloning vector according to manufacturer's instructions (Promega, Madison, WI). Plasmid DNA was isolated by the

alkaline lysis method [22]. Plasmids were screened for the appropriate size insert by agarose gel electrophoresis.

PCR cycle sequencing of cloned inserts was performed using the ABI PRISM DNA sequencing kit in conjunction with an ABI 377 automated DNA sequencer (Perkin Elmer, Foster City, CA).

Clones from each environmental library were screened by sequencing approximately 450 base pairs using the universal rRNA primer 357F (5'-CTA CGG GCG CAG CAG-3'). Screened sequences were compared for sequence similarity Those with 98% or greater identity were grouped, and one was chosen as a group representative for full-length sequencing of the cloned DNA insert.

Phylogenetic Analysis

Full-length sequences were initially aligned with related Ribosomal Database Project (RDP) sequences using the programs SIMILARITY RANK and ALIGN [15] and then manually aligned. Aligned sequences were analyzed for chimera formation, after which phylogenetic analysis was performed using maximum likelihood, similarity matrix and maximum parsimony analyses [7, 15].

Results

Nutrients and Oxygen Levels in Interstitial Water

The fertilizer applications were successful at delivering fertilizer nutrients to the interstitial water of the oiled zone, both in August and October. Only very minimal levels of fertilizer nutrients were detected in the untreated or tilled plots, or in nearshore water. These values were very close to the detection limits of the test kits used here.

The application of fertilizer resulted in depressed oxygen levels in August and October, suggestive that aerobic metabolism of the indigenous organisms was stimulated by fertilizer application. The indigenous bacteria are thus well adapted to the psychrophilic conditions existing on the beach in October. It is important to note, however, that the dissolved oxygen levels never fell below 2 ppm oxygen (approximately 25% of saturation under these conditions), so the beach remained aerobic despite the increased microbial activity.

Analysis of Total Biomass

Phospholipids are part of the intact cell membranes and provide a quantitative measure of microbial biomass [30, 2]. The phospholipid content of sediment samples from the oiled beach sites and the remote un-oiled site is shown in Table 2. Comparison of total phospholipid shows a markedly higher concentration of biomass on the oiled and fertilized beach and a slightly elevated content on the oiled, tilled and fertilized beach. This trend is seen in both the prokaryotic and eukaryotic phospholipid markers, suggesting that fertilizer addition stimulated the growth of both communities.

Metabolic Status

In Gram negative bacteria, the monoenoic fatty acids (16:1w7c & 18:1w7c) are converted to cyclopropyl fatty acids (cy17:0 & cy19:0) as microbes move from a logarithmic to a stationary phase of growth (i.e., slow their growth) [28]. This change is expressed in the two ratios cy17:0 /16:1ω7c and cy19:0/18:1ω7c. The ratios are quite variable, but usually

Table 2. Phospholipid fatty acid analysis of sediment samples.

Beach	Picomoles PFLA/g dry sediment			Sum of cyclopropyl fatty acid ratios
	Total	Prokaryotic	Eukaryotic	
Un-oiled Control	2,467	2,346	121	0.12
Oiled Untreated	2,062	1,988	74	0.12
Oiled & Tilled	1,296	1,267	29	0.39
Oiled & Fertilized	13,386	12,355	1,029	0.02
Oiled, Tilled & Fertilized	4,634	4,386	248	0.04

fall within the range of 0.05 (log phase) to 2.5 (stationary phase). When the ratios are summed the range is from 0.1 to 5.0, and is inversely proportional to the turnover rate (i.e., a lower ratio implies a higher turnover rate). The data in Table 2 indicate that the highest metabolic activity was found in fertilized beach sediments consistent with the levels of biomass observed. The tilled un-fertilized beach had the highest ratio indicative of the lowest metabolic status of the Gram negative bacterial community.

PCR Analysis of Catechol 2,3 Dioxygenase

C23O catalyzes an important ring opening reaction in the catabolic pathway of aromatic hydrocarbons, and it can be used as the target for analysis of aromatic hydrocarbon degradation [24, 31]. Many oil-degrading organisms produce C23O, which converts catechols to 2-hydroxymuconic semialdehydes. C23O is important in the degradation pathway of naphthalene and other polynuclear aromatics as well. The gene coding for the enzyme C23O is encoded by the *nahH* gene found on plasmid NAH7 of *Pseudomonas putida*. A similar C23O enzyme coded by the gene *xylE* of the TOL plasmid WWO has 81% sequence homology and 85% amino acid homology to the protein product coded for by *nahH* [3].

DNA extracted from the oiled and un-oiled sediment samples was PCR amplified using primers specific for the C23O gene (*xylE/nahH*). While amplifiable DNA was obtained from all samples, the gene for C23O was only detected in DNA isolated from fertilized and tilled/fertilized samples and not in the others. This indicates that fertilizer addition stimulated the population of organisms possessing this gene and suggests that without fertilizer addition, growth on aromatic hydrocarbons was nutrient limited.

Phylogenetic Analysis

16S ribosomal RNA gene based phylogenetic analysis was performed on a composite sample from the untilled fertilized section of the oiled test beach (oiled sediment sample) and on a composite sediment sample taken from a remote un-oiled site approximately 300 m from the test site. Fifty one 16S clones were sequenced from the oiled sediment sample and 34 unique sequences identified (67%). Seventy-one clones were sequenced from the un-oiled control sample and 51 unique sequences representing 72% of the total were identified. The phylogenetic affiliations of the rDNA clones identified in this study are shown in Table 3. 16S clones most closely related to the Gamma *Proteobacteria* were the largest group, and clones placed within subdivision I of the *Flexibacter-Cytophaga-*

Bacteroides phylum were the second largest group, in both oiled and un-oiled sediment samples. Combined, the clones placed in these two groups accounted for 73 and 80% of the total clones from the un-oiled and oiled beach sediment, respectively. In addition, the un-oiled beach sediment contained a significant portion of clones (11% of the total) most similar to members of subdivision II of the *Flexibacter-Cytophaga-Bacteriodes* phylum. A number of clones with greater than 98% similarity to *Cycloclasticus pugetti* were identified in the oiled and fertilized sediment sample and a single clone with 99% similarity to *Yersinia rohdei* was isolated from the un-oiled beach sediment. While none of the identified sequences were identical to sequences in public databases, all of the gram negative clones had greater than 85% similarity to 16S sequences from previously isolated organisms and could be placed in the defined taxonomic groups indicated. The gram positive clones (5% of the total from the un-oiled beach, none detected on the oiled beach) had sequence similarities between 80 and 85 % to reported sequences and could not be confidently placed in currently recognized divisions. Unclassified organisms (4% of sequences from both oiled and un-oiled samples) had less than 80% similarity to reported sequences and seem to be unaffiliated with known bacterial lineages.

The distribution of Gamma *Proteobacteria* clones from un-oiled and oiled sediments is shown in Fig. 2. The difference in the number of clones between the two beaches can largely be accounted for by the presence in the oiled beach sediment of abundant sequences from two phylogenetic groupings that were not identified in the un-oiled sediment sample. The first group, representing 20% of the total clones, places with *Pseudomonas* and relatives, and included clones most similar to members of the *Acinetobacter* subgroup (*Acinetobacter johnsonii*, 92% similarity, *Moraxella catarrhalis*, 91% similarity), *Teredinibacter* subgroup (strain SCB111, 88% similarity), and the *Pseudomonas* subgroup (*Pseudomonas flavescens*, 95-98% similarity). The second group of clones, representing 18% of the total, had greater than 98% sequence similarity to *C. pugetti*, located by 16S phylogenetic relationship in the *Thiomicrospira* assemblage. Together these two groups

Table 3. Phylogenetic affiliations of identified rDNA sequences.

Phylogenetic group	**Percent of Total Clones**	
	Un-oiled Beach	**Oiled Beach**
Gamma *Proteobacteria*	39	60
Flexibacter-Cytophaga-Bacteroides phylum Subdivision I	34	20
Flexibacter-Cytophaga-Bacteroides phylum Subdivision II	11	0
High G+C Gram positive bacteria	4	0
Delta *Proteobacteria*	3	4
Leptospirillum group	3	2
Green non-sulfur bacteria	1	0
Beta *Proteobacteria*	1	0
Alpha Proteobacteria	0	6
Epsilon *Proteobacteria*	0	4
Unclassified	4	4

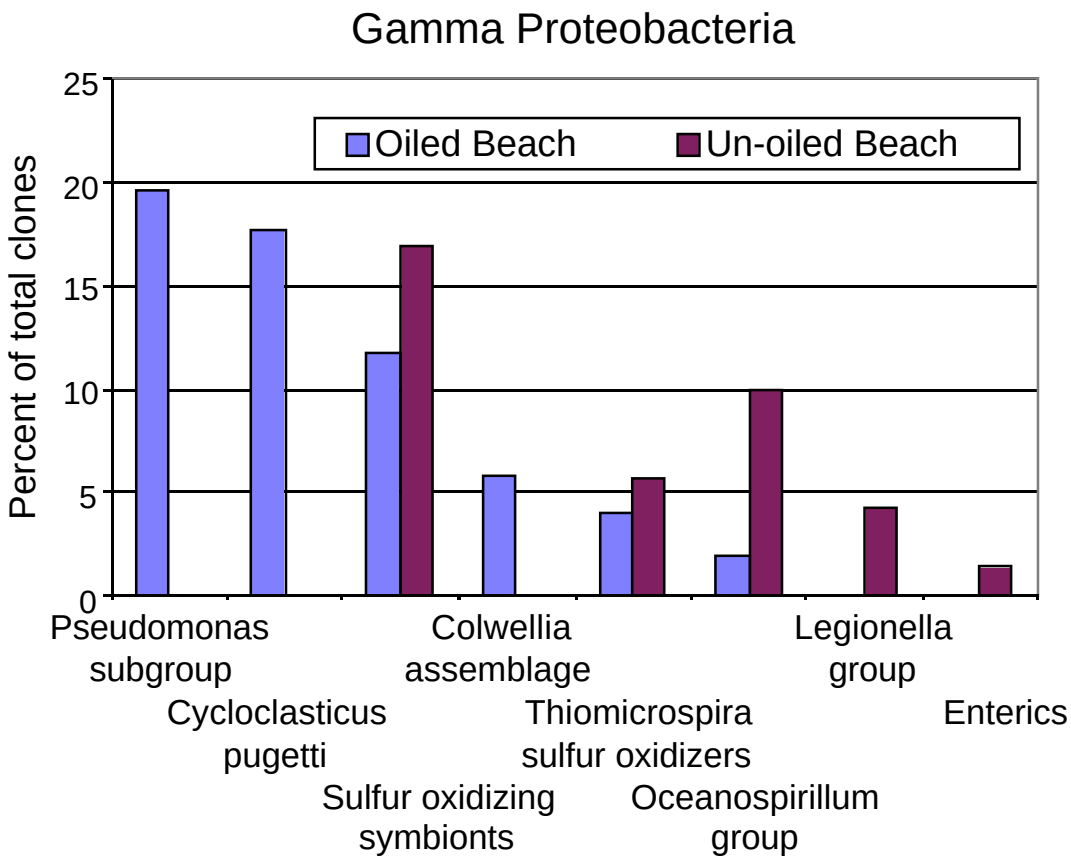

Fig. 2. Comparison of Gamma *Proteobacteria* groups. *Cycloclasticus pugetti* is placed within the *Thiomicrospira* assemblage in the sequence database of the Ribosomal Database Project.

Dyksterhouse et al. [6] isolated *C. pugetti* by growth on biphenyl from marine sediments collected in Puget Sound. Related organisms have been detected in hydrocarbon contaminated and noncontaminated marine sediments in Puget Sound and in sediments from Resurrection Bay off the Gulf of Alaska [9, 8, 29]. *C. pugetti* grows on a variety of aromatic hydrocarbons including biphenyl, naphthalene, phenanthrene, anthracene and toluene. However, unlike other members of the *Thiomicrospira* assemblage, *C. pugetti* does not use thiosulfate as an energy source, and is believed to represent a novel genus [6].

Members of the Acinetobacter subgroup, which includes the genera *Acinetobacter* and *Moraxella*, and the genus *Pseudomonas* are frequently associated with hydrocarbon degradation [11, 21, 17].

The most abundant Gamma-*Proteobacteria* sequences identified in the un-oiled sediments were most similar to the sulfur-oxidizing gill symbionts of marine bivalves (17% of the total clones) and these sequences were also highly represented in the oiled sediment (12% of the total clones). Clones most similar to symbionts from *Solemya reidi*, *Codakia orbicularis*, *Anodontia phillipiana* and *Thyasira flexuosa* were identified with sequence similarities ranging from 87-92%. The clones identified may represent free-living relatives of these symbiont organisms. Clones that cluster with the sulfur-oxidizing gill symbionts were also identified in marine sediments from Puget Sound [9]. Another group of clones, most similar to sulfur oxidizing bacteria located in the *Thiomicrospira* group (excluding the

C. pugetti discussed above), was also identified in the oiled and un-oiled sediment, representing 4 and 6% of the total clones respectively.

Gamma-*Proteobacteria* clones placed in the marine assemblage *Oceanospirillum* represented 10% of the total clones from the un-oiled sediment and 2% from the oiled sediment and were most similar to *Oceanospirillum linum* (90% similarity) and *Oceanospirillum kriegi* (95% similarity) respectively. The remaining oiled beach sediment Gamma-*Proteobacteria* clones, representing 6% of the total, were most similar to the Pacific Ocean phytodetrital marine snow associated clone env. agg53 (94-95% similarity), located in the *Colwellia* assemblage [5]. The remaining Gamma-*Proteobacteria* clones from the un-oiled beach sediment (4% of the total clones) were most similar to members of the *Legionella* group (*Legionella lytica*, 91% similarity, *Coxiella burnettii*, 92% similarity, and the enteric bacterium *Yersinia rohdei* (99% similarity, represented by a single clone). The distribution of clones most similar to members of the *Flexibacter-Cytophaga-Bacteroides* phylum from the un-oiled and oiled sediments are shown in Fig. 3. All but one of the clones were most similar to reported sequences from the genera *Cytophaga*, *Flexibacter*, *Flavobacterium*, and *Saprospira*. Surface-dependent gliding is common among these organisms, as is the degradation of a wide range of biopolymers including cellulose, starch, chitin, proteins and nucleic acids.

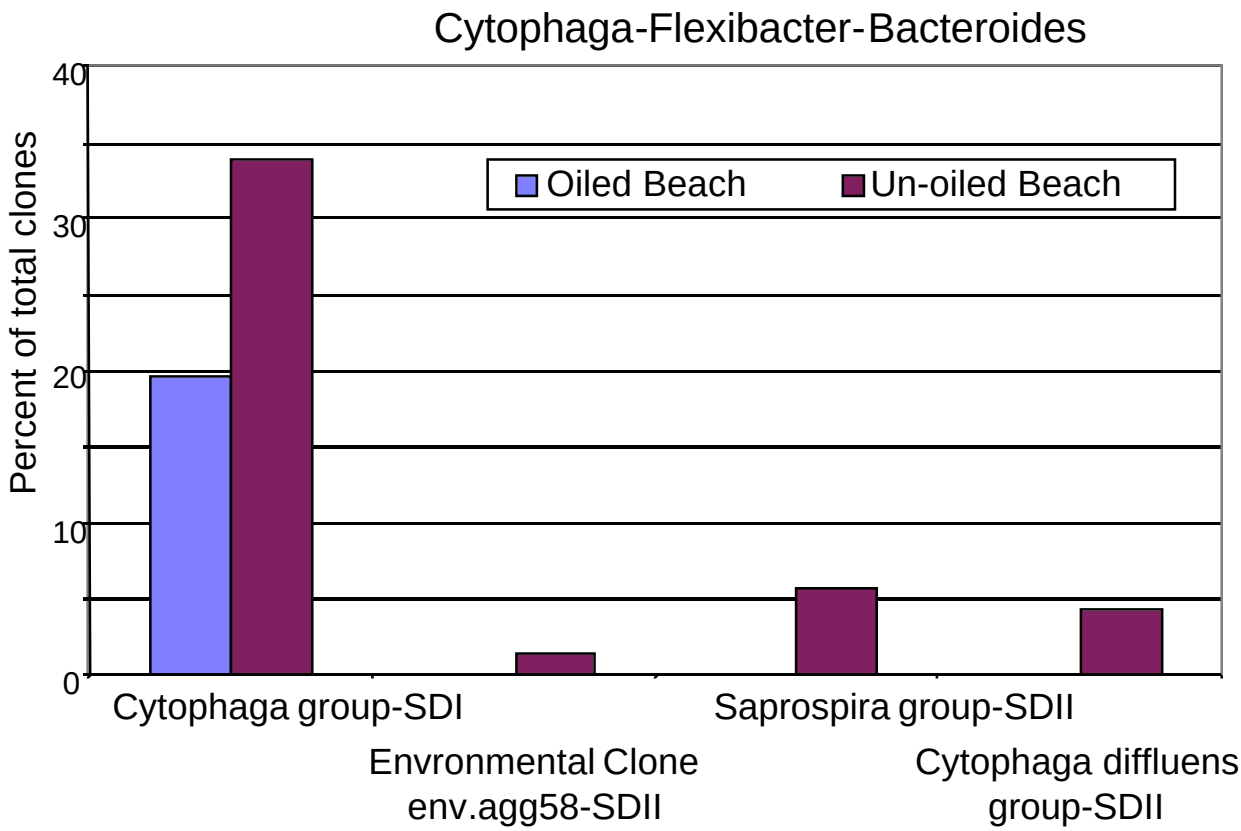

Fig. 3. Comparison of *Flexibacter-Cytophaga-Bacteroides* phylum clones obtained from the oiled and un-oiled sediments.

The majority of the *Flexibacter-Cytophaga-Bacteroides* phylum clones from both sediments could be grouped in the *Cytophaga* group in subdivision I of this phylum, representing 34% of the total clones form the un-oiled beach sediment and 20% from the oiled beach sediment. Of these clones, 50% from the un-oiled beach sediment and 100% from the oiled beach sediment were most closely related to the Pacific Ocean phytodetrital marine snow associated clone env.agg13 (91-94% similarity), located in the *Cytophaga lytica* subgroup [5]. The remaining un-oiled beach *Cytophaga* group clones also placed in the *Cytophaga lytica* subgroup, with 25% most similar to *Cytophaga uliginosa* (89-94% similarity), 13% to *Flavobacterium salegens* (92% similarity) and 12% to *Flexibacter maritimus*. (90% similarity). The remaining members of the *Flexibacter-Cytophaga-Bacteroides* phylum from the un-oiled beach sediment, representing 11% of the total clones and 24% of the clones placed in the *Flexibacter-Cytophaga-Bacteroides* phylum, were grouped in subdivision II. Of these clones, 55% were most similar to *Cytophaga diffluens* (86% similarity), 36% to the marine snow associated clone env.agg32 (89% similarity) located in the *Saprospira* group [5], and 10%, represented by a single clone, could not be placed with confidence but had a 82% similarity to the marine snow associated clone env.agg58 [5].

The remaining taxonomic groups identified in both samples make up relatively minor components of the total clone population (see Table 3.). Perhaps the most distinguishing differences between the two environments is the identification of Alpha- and Epsilon-*Proteobacteria* in the oiled but not in the un-oiled sediment. Of the Alpha-*Proteobacteria* clones, two were most similar to the *Rhodospirillum rubrum* assemblage and one to the *Rhodobacter* group. The identified Epsilon-*Proteobacteria* were placed with *Campylobacter* and relatives, whose members are characterized as chemoorganotrophs that do not oxidize carbohydrates.

Conclusions

Phospholipid fatty acid, gene probe and 16S microbial community analysis suggest that bioremediation stimulated the metabolic activity, increased microbial biomass and genetic potential for aromatic hydrocarbon degradation, and increased the population of hydrocarbon degradation associated members of an oiled Arctic shoreline microbial community.

Stimulation of metabolic activity is supported by the ratio of the monoenoic fatty acids (16:1ω7c and 18:1ω7c) to cyclopropyl fatty acids (cy17:0 and cy19:0) expressed as the sum of the two ratios cy17:0 /16:1ω7c and cy19:0/18:1ω7c. This sum is inversely proportional to the turnover rate (i.e., a lower value infers a higher turnover rate). The tilled un-fertilized beach had the lowest metabolic status, while the highest metabolic activity was found in fertilized beach sediments.

The level of total phospholipid fatty acid in the sediments indicated increased biomass. Fertilized beach sediments had substantially more biomass than un-fertilized and un-oiled sediments.

Increased genetic potential for aromatic hydrocarbon degradation is supported by the ability to detect C23O genes in sediments from fertilized sites and not in sediments from the unfertilized and un-oiled beaches.

Changes in the microbial community were detected by 16S ribosomal DNA analysis. While only 122 total clones were sequenced, clear differences were observed between the un-oiled and oiled and fertilized beach sediments. The major difference was that a substantial proportion of the community detected in the bioremediated oiled beach sediment could be reasonably associated with known hydrocarbon degrading bacteria; e.g., *Acinetobacter, Moraxella* and *Cycloclasticus*. These organisms were not detected in the un-oiled beach sediment.

Although based on a small number of samples, taken together these data are fully consistent with the expectations that are the basis for nutrient assisted bioremediation; hydrocarbon degrading microbes make up only a small subset of the total community that persists at very low levels until stimulated by the presence of oil. In the case of these sediments, this microbial community appears to consist largely of members of the *Flexibacter-Cytophaga-Bacteroides* phylum and the Gamma *Proteobacteria*. The presence of oil and fertilizer encourages the growth of oil degrading bacteria, which in this case largely appear to be in the Gamma *Proteobacteria* distinct from those present in the absence of oil.

Our data indicate that bioremediation stimulated the hydrocarbon utilizing population in this Arctic shoreline microbial community. Overall, the results from this study are consistent with the stimulation of oil biodegradation observed in sub-arctic marine environments [16, 20, 4, 13] and suggest that biodegradation of oil spills in Arctic environments can also be stimulated by fertilizer addition.

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