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The Importance of Weathered Crude Oil as a Source of Hydrocarbonoclastic Microorganisms in Contaminated Seawater

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This study investigated the hydrocarbonoclastic microbial community present on weathered crude oil and their ability to degrade weathered oil in seawater obtained from the Gulf St. Vincent (SA, Australia). Examination of the native seawater communities capable of utilizing hydrocarbon as the sole carbon source identified a maximum recovery of just 6.6×10^1 CFU/ml, with these values dramatically increased in the weathered oil, reaching 4.1×10^4 CFU/ml. The weathered oil (dominated by $>C_{30}$ fractions; 750,000 ± 150,000 mg/l) was subject to an 8 week laboratory-based degradation microcosm study. By day 56, the natural inoculums degraded the soluble hydrocarbons (initial concentrations 3,400 ± 700 mg/l and 1,700 ± 340 mg/l for the control and seawater, respectively) to below detectable levels, and biodegradation of the residual oil reached 62% (254,000 ± 40,000 mg/l) and 66% (285,000 ± 45,000 mg/l) in the control and seawater sources, respectively. In addition, the residual oil gas chromatogram profiles changed with the presence of short and intermediate hydrocarbon chains. 16S rDNA DGGE sequence analysis revealed species affiliated with the genera *Roseobacter, Alteromonas, Yeosuana aromativorans,* and *Pseudomonas*, renowned oil-degrading organisms previously thought to be associated with the environment where the oil contaminated rather than also being present in the contaminating oil. This study highlights the importance of microbiological techniques for isolation and characterisation, coupled with molecular techniques for identification, in understanding the role and function of native oil communities.

Keywords: Weathered crude oil, biodegradation, DGGE, hydrocarbonoclastic, Pareto–Lorenz curve

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Oil spills occur in different environments including soil, freshwater, and marine waters. Each type of environment represents a unique ecosystem with different resources, community structure, and degradation potential. The natural presence of hydrocarbonoclastic microorganisms plays a critical role in the natural attenuation of polluted environments [22]. In some cases, the native hydrocarbonoclastic community is more effective in degrading the hydrocarbon in that particular environment than any introduced organism, as not all hydrocarbonoclastic bacteria are capable of degrading hydrocarbons in every ecosystem.

It has been estimated that 80% of the maritime traffic is in the northern hemisphere [8]. In addition, the drilling industry also has large activity in this region including the Arabian Gulf, North Sea, and Gulf of Mexico. Therefore, marine oil spills in the northern hemisphere are more common than in the southern hemisphere and as a result are highly publicized including the *Exxon Valdez* (1989), *Prestige* (2002), and recently the *Deepwater Horizon* spill in the Gulf of Mexico (2010); in contrast southern hemisphere oil spills are less well known but include *Princess Anne Marie* (1975), *Kirki* (1991) [2], and the *PTTEP West Atlas* (2009) off the Australian western coast, and most recently *Rena* (2011) in the Bay of Plenty, New Zealand (NZ). Despite the hemisphere's lower activity, biodegradation experiments in all regions need to be studied to ensure that adequate hydrocarbon degradation distinct strategies are developed.

Following the introduction of oil into aquatic environments, many indigenous hydrocarbon-degrading microorganisms have been identified including marine bacterial species affiliated with the genera *Alcanivorax*, *Marinobacter*, *Thallassolituus*, *Cycloclasticus*, and *Oleispira* [24]. The vast diversity of hydrocarbon-degrading organisms in the aquatic environment has been revealed by microbial isolation and cultivation from both oil and water samples [17]. However, the role that natural oil microflora play in contributing to the active hydrocarbonoclastic community

of the environment where the oil has been introduced into has rarely been examined.

In addition to the examination of the hydrocarbonoclastic community, it is also important to be able to assess the impact of the contamination or the intervention on the natural microbial community. In this case, recent research has focussed on the development and application of molecular microbial ecology techniques such as the community fingerprinting technique denaturant gradient gel electrophoresis (DGGE) together with real-time PCR (RT-PCR) [16]. The application of these molecular ecology techniques provide more precise perspectives of the diversity between microbial communities [17], usually targeting the 16S rDNA gene [4]. Clearly one conclusion from the molecular microbial ecology work that has been carried out in the last decade is that although there has been a range of bacteria isolated, compared with the overall diversity of organisms identified (but not cultured) using molecular techniques, this is actually a very small percentage of the community [17]. Sequencing databases are rapidly growing [7] as are the numbers of uncultured organisms being identified.

The main objective of this study was to use microbiological techniques to establish the diversity of the hydrocarbonoclastic community naturally present on weathered crude oil and to use molecular techniques to determine the impact they have on aquatic environments contaminated with weathered crude oil. Therefore, the study described aimed to (i) examine the microbial community present in the weathered crude oil; (ii) analyze the hydrocarbonoclastic community potential of seawater to degrade weathered crude oil, and (iii) analyze the community structure using molecular biological techniques targeting the 16S rDNA gene.

Materials and Methods

Sample Collection

Seawater samples were collected from the Gulf St. Vincent (SA, Australia) and filtered (10 micron). Sterile non-saline water was used as the control water source (0.22 micron membrane filter). The weathered crude oil used in this study was obtained from an oil refinery (SA, Australia).

Enumeration of the Hydrocarbonoclastic Population Present in Weathered Crude Oil

Enumeration of the microbial population in seawater was carried out by serially diluting the water phase by taking an aliquot of the sample, undiluted (100 µl), or transferring an aliquot (10 µl) to sterile rich broth (990 µl, Difco 2216; France) for dilution (1:100) and repeating consequently for further dilutions (1:1,000). After homogenization, an aliquot (100 µl) was spread over either media rich (marine agar, MA) plates (Difco 2216, France), for the determination of the total recoverable bacterial population or onto minimal salt media (MSM) plates [3] with the addition of 2% agar (Oxiod, LP0013) and 0.2% oil as the sole carbon source [9], for total hydrocarbon-degrading population and incubated at 25°C for 2–14 days.

The numbers of hydrocarbonoclastic organisms and total extractable microbial populations present in the weathered crude oil were also determined. The weathered crude oil was vigorously mixed using a vortex with sterile molecular grade water (20 ml sterile water/1 g oil) to transfer the cells into the aqueous phase to allow enumeration of the microbial populations, which was carried out as described above.

Weathered Crude Oil Microcosm Experiment

Experiments on the degradation of weathered crude oil were carried out in replicated microcosms (n = 3, each with 500 ml volume) containing the water sample (200 ml). Weathered crude oil was added [1% (v/v)] and the aerobic microcosms were incubated at 25°C (in natural daylight), with an aeration of 120 rpm for 8 weeks (56 days).

TPH Concentration Analysis

Quantitative and qualitative analyses of the total petroleum hydrocarbon (TPH) concentration were carried out at the initial and final stages of the experiment (Day 0 and Day 56). For the soluble hydrocarbon concentrations in the aqueous phase, hydrocarbons in replicate water samples were extracted using solid phase extraction (SPE) columns [5] as described by the manufacturer's methods (Phenomenex, USA). Briefly, samples (20 ml) were transferred to the SPE tubes, and the extracts were eluted with hexane:dichloromethane (1:1) to obtain aliphatic and aromatic fractions. The extracts were analyzed using an 8200 Autosampler gas chromatograph with a 261 Varian 8200 Autosampler with flame ionization detector (FID) and mass spectrometry (MS) detector [22]. In addition, the initial and residual weathered crude oil total petroleum hydrocarbon (TPH) concentrations were extracted and analyzed by Flinders Advanced Analytical Laboratory, Flinders University (ISO accreditation 17025 for TPH). Briefly, 1 g of oil was extracted in a "liquid–liquid" manner. Solvent (1 ml) was analyzed *via* GCMS [22]. TPH is defined as the area under the GC-FID chromatogram between the elution of *n*-$C_{10}H_{22}$ and *n*-$C_{40}H_{82}$. It is calibrated using a known amount of a solution of diesel and motor oil.

DNA Extraction

Genomic DNA was extracted from the water microcosms using a modified method from McKew *et al*. [15]. Bacterial community samples were transferred to a sterile 2 ml tube containing 0.5 g of glass beads (size 212–300 µm) and equal volumes of phenol-chloroform-isoamyl alcohol (25:24:1). The cells were lysed by bead beating for 2 × 20 s (Mini Bead Beater K9, BioSpec), with samples kept on ice in between beats. Following bead beating, samples were centrifuged at 12,000 ×*g* for 5 min at 4°C. The upper phase was removed into a sterile tube containing equal volumes of phenol-chloroform-isoamyl alcohol and again centrifuged at 12,000 ×*g* for 5 min at 4°C. The upper phase was removed and further purified with equal volumes of chloroform-isoamyl alcohol (24:1). The DNA was precipitated by adding an aliquot (70 µl) of sodium acetate (3 M) and ice-cold filter sterile isopropanol (1 ml), which was incubated at −20°C for ≥ 2 h. After incubation, the precipitated DNA was centrifuged, washed with 70% ethanol, and air dried. After evaporation of the remaining ethanol solution, molecular-grade water (30–50 µl) was added to resuspend the DNA. Community DNA extractions were performed in replicates at selected time

points (weeks 0, 2, 4, 6, and 8) to analyze the changes in microbial (bacteria) community profiles.

Microbial Community Structure

Polymerase chain reaction (PCR) amplification of bacterial 16S ribosomal DNA (rDNA) was performed in a PCR reaction mixture (50 µl) containing forward primer 341F with GC Clamp (2 µl, 10 pmol/µl), reverse primer 518R (2 µl, 10 pmol/µl) [16], magnesium chloride (5 µl, 25 mM), deoxynucleoside triphosphate (dNTP) mixture (1 µl, 10 mM), GoTaq Flexi buffer (10 µl, 5 ×), Taq polymerase enzyme (0.25 µl, 5 U/µl), and sterile nuclease-free water (27.75 µl). For each sample, purified template DNA extract (2 µl) was added to the PCR master mix (48 µl). Cycle conditions used for bacterial DNA amplification were 1 cycle of 5 min at 95°C, followed by 4 cycles of 35 s at 94°C, 30 s at 55°C, and 1 min at 72°C. This was followed by 25 cycles of 30 s at 92°C, 30 s at 55°C, 1 min at 72°C, and a final extension at 72°C for 10 min and 4°C for 1 min. For each set of reactions, a negative control in which template was replaced with sterile molecular-grade water was included. The PCR products were validated by a 1.5% agarose gel electrophoresis.

The changes in microbial community composition were monitored using DGGE. PCR amplicons were analyzed on a D-Code apparatus (Bio-Rad, USA) on a polyacrylamide gel (acrylamide–*N*,*N*'-methylenebisacrylamide ratio 37:1) using urea formamide. Electrophoresis was carried out for 18 h at 60°C and a constant voltage of 60 V with a linear denaturant gradient of 47–62% for universal bacterial community analysis [22]. After electrophoresis, the gel was stained by silver staining, scanned using an Epson V700 scanner, and the bacterial community profile analyzed [22].

Selected DGGE bands were excised and incubated in elution buffer [15]. Re-amplification was performed by PCR as described previously, except forward primer without the GC clamp was used. Products were purified using the Wizard SV Gel and PCR Clean-up System (Promega, USA) as per the manufacturer's protocol. Products were then sent for sequencing (Australia Genome Research Facility, Adelaide, Australia).

Statistical Analysis

The digitized DGGE image was analyzed using the Phoretix 1D advanced analysis package (Phoretix Ltd, UK). The Phoretix package was used to study the relatedness of the microbial communities being expressed as similarity coefficients [13]. An unweighted pair group method using arithmetic mean algorithm (UPGMA) dendrogram was constructed from Dice–Sorensen's similarity matching index as described by Patil *et al*. [18]. The algorithm used the equation $S_{J(K)} = (1/t_J t_K)(\sum_{SJ(K)})$, where the similarity between a sample and an existing cluster equals the arithmetic mean of similarities between the sample and all members of the cluster [18]. The Shannon index (H') was calculated from DGGE profiles using the formula $H' = -P_i LNP_i$ [7]. Pareto–Lorenz (PL) curves were used to estimate functional organization and evenness within the microbial community [14]. Each band was considered to be an operational taxonomic unit (OTU) or species and the band densities were then used to calculate the PL curve. For each DGGE lane, the respective bands were ranked based on their intensities from highest to lowest. Subsequently, the cumulative normalized number of OTUs was used as the X-axis, and the respective cumulative normalized intensities were represented on the Y-axis. Statistical significance was determined using paired t-tests performed in statistical package SPSS (PASW Statistics 18).

Table 1. Comparative cell counts (CFU/ml) between rich media and minimal salt media types in seawater and on weathered crude oil.

Sample	Marine agar (SE n=3)	Minimal salt media (SE n=5)
Seawater	5,153 ± 165	66 ± 3
Weathered crude oil	42,110 ± 473	41,200 ± 511

RESULTS

Hydrocarbonoclastic Community Present in the Weathered Crude Oil

Bacteria capable of using crude oil as the sole carbon source were enumerated by plating seawater on minimal salt media agar plates containing crude oil. The hydrocarbonoclastic microbial population was compared with the total recoverable population, which were isolated on rich media. The results shown in Table 1 demonstrate that minimal hydrocarbon degraders were naturally present in the seawater sources, with 1.2% of the total recoverable seawater community able to utilize the crude oil.

Enumeration of the cultivable hydrocarbonoclastic bacteria naturally present on the crude oil exceeded those from the seawater sources (Table 1). Of the total recoverable weathered crude oil community, 97.8% were able to utilize the hydrocarbon source. These results highlight the selectivity and abudance source of hydrocarbonoclastic organisms present naturally on weathered crude oil.

Total Petroleum Hydrocarbon Degradation

Gas chromatography equipped with mass spectrometry established the crude oil as a weathered oil by the

Table 2. Total petroleum hydrocarbon (mg/l) concentrations and degradation rates (%) of the water column and the residual crude oil from the seawater and control microcosms.

	Water column TPH			Oil TPH		
Sample	Initial	Final (Day 56)	% Degraded	Initial	Final (Day 56)	% Degraded
Control	3,400 ± 700	< 100	100#	750,000 ± 150,000	285,000 ± 45,000	62
Seawater	1,700 ± 340	< 100	100#	750,000 ± 150,000	254,000 ± 40,000	66

means approximation.

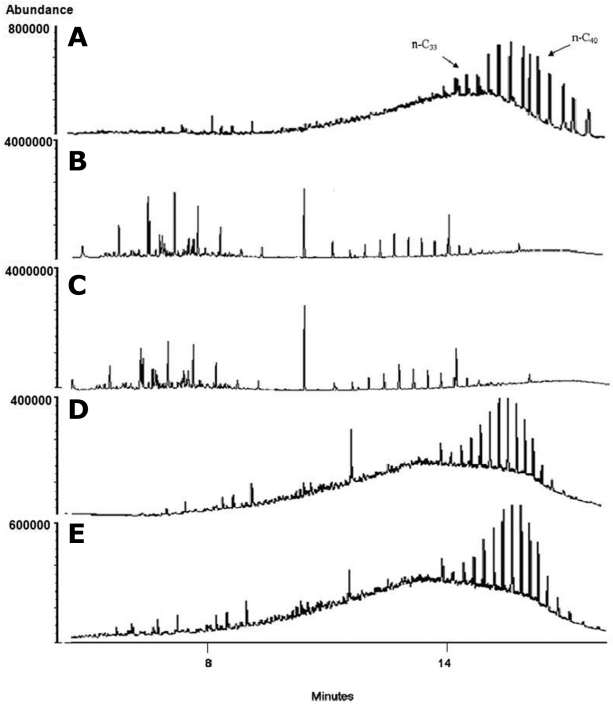

Fig. 1. Total petroleum hydrocarbon chromatograms of the (**A**) initial weathered crude oil, (**B**) seawater from water column (Day 0), (**C**) control water from water column (Day 0), (**D**) seawater from residual weathered crude oil (Day 56), and (**E**) control water from residual weathered crude oil (Day 56).
The arrows indicate the position of the n-C_{33} and n-C_{40} fractions as reference peaks.

depletion of lower chain hydrocarbons and strong presence of high chain (>32C) hydrocarbons (Fig. 1). Microcosm experiments following both the fate of the weathered crude oil and its impact on the microbial community were examined over an 8 week time period. At time zero, the total petroleum hydrocarbon (TPH) concentration of the water microcosms contaminated with weathered crude oil were approximately 1,700 ± 340 mg/l for the seawater and 3,500 ± 700 mg/l for the control water (Table 2).

Throughout the experiment, emulsification of the hydrocarbon and colour (yellow) pigmentation were visible in some microcosms, signifying initial signs of degradation [15], production of intermediate products as a result of the breakdown of the hydrocarbons, or proliferation of hydrocarbon-degrading organisms. By the conclusion of the experiment, the levels of TPH in the water samples had reduced significantly (Paired T Test, $P<0.05$). The TPH concentration in the microcosms was reduced to levels below the detectable level (<100 mg/l). These results confirmed that the oil fractions that were soluble in the water were significantly degraded, confirming the presence of active hydrocarbonoclastic bacteria.

In addition to the water TPH concentration, the oil fraction was analyzed on GC/MS and the concentrations are shown in Table 2. By Day 56, the total petroleum hydrocarbon concentration of the residual oil in seawater reduced 66% to 254,000 ± 40,000 mg/kg. The TPH concentration of the residual oil in the control reduced 62% in the 8 weeks to 285,000 ± 45,000 mg/kg.

The chromatogram results (Fig. 1) show the changes in the oil profile between the initial and final chromatograms of the weathered oil. Fig. 1A shows the initial weathered crude oil with a dominance of high chain hydrocarbons. In Fig. 1D and 1E, there was a shift in the high chain

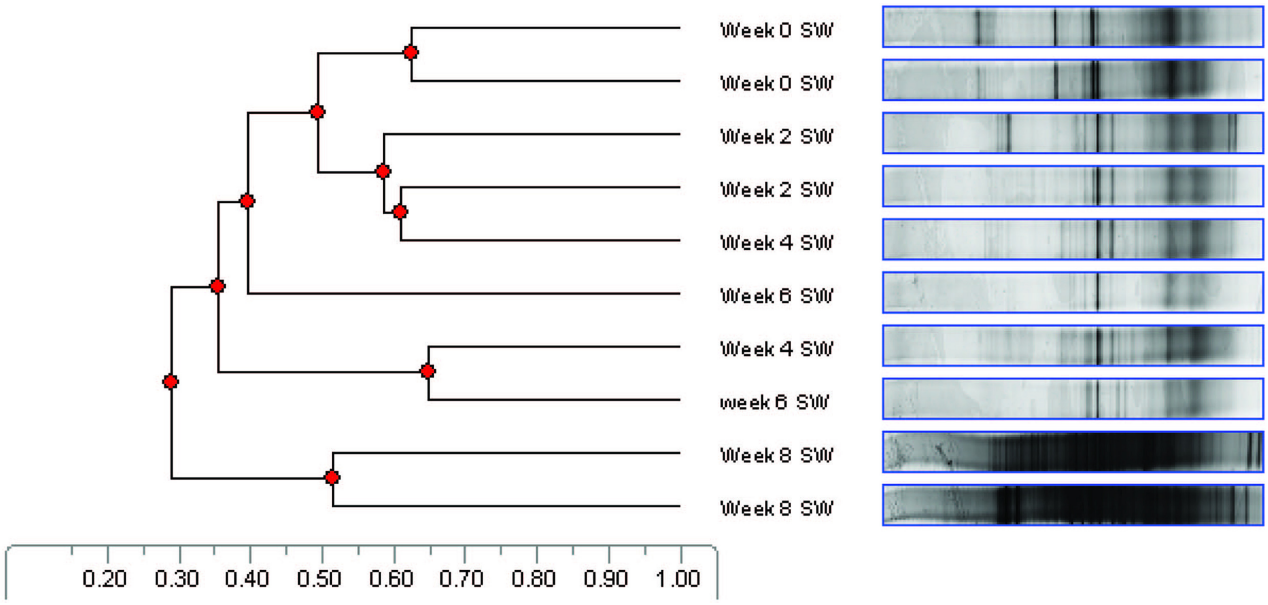

Fig. 2. UPGMA (Unweighted Pair Group Method with Arithmetic mean algorithm) dendrogram of seawater (SW) + oil samples.
The scale represents a unitary scale of similarity (Dice–Sorensen's similarity index).

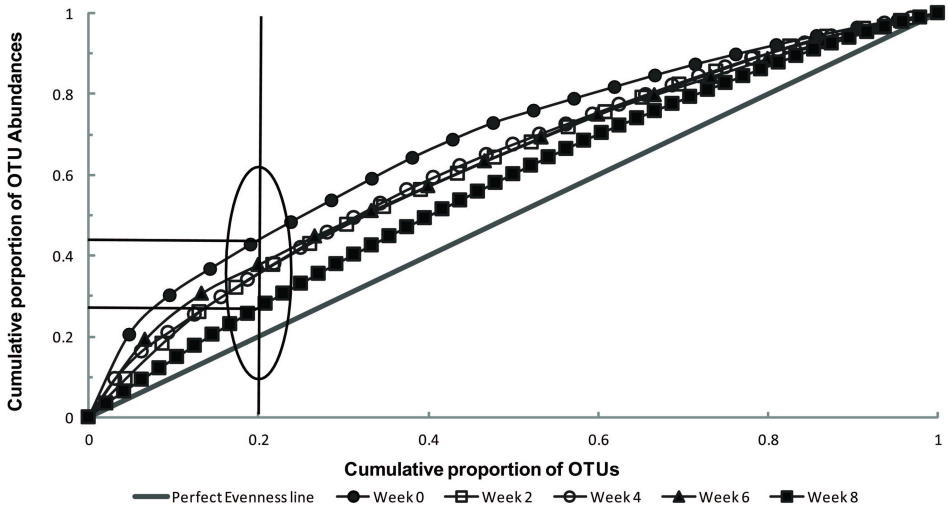

Fig. 3. PL curve representing perfect evenness line and week 0 to week 8 from the seawater microcosms.
Duplicate samples were analyzed.

hydrocarbons dominance with the addition of intermediate length hydrocarbons and an increase in short chain hydrocarbon peaks by day 56.

Molecular Analysis (PCR and DGGE)

Having established that the hydrocarbonoclastic organisms were present and actively degrading, molecular techniques were used to determine the effects an oil spill of crude oil would have in the marine environment.

The seawater microcosm community is shown in Fig. 2. The UPGMA (Unweighted Pair Group Method with Arithmetic mean algorithm) dendrogram shows a main cluster formed primarily in relation to time. The bacterial diversity values over the first 6 weeks were similar (week 0; H' 2.49) with an increased diversity observed in week 8 (H' 3.44). In addition, the time-related trend was also seen in the Pareto–Lorenz curve (Fig. 3). The PL curve distribution patterns were used to assess the evenness and functional organization based on the cumulative number of bands and their intensities [1]. The PL curve of weeks 0, 2, 4, and 6 were in the 45% PL curve area, from approximately 35% to 43%. The PL curve of week 8 is referred to the 25% curve, as it intercepts the 20% X-axis at 25% abundance, which indicates a highly even community profile.

The microbial profiles were also assessed by 16S sequence analysis of excised DGGE bands. Bands were identified on the basis of their 16S rDNA gene homology with entries in the GenBank database using the BLAST alignment tool. Accession numbers for isolate sequences and the level of similarity to related organism are shown in

Table 3. Phylogenetic affiliation of 16S rRNA gene sequences obtained from DGGE gel.

Sample	Closest relatives in GenBank database	Phylogenetic class	Phylum	Similarity (%)
Seawater	*Lysinibacillus sphaericus* (HM125962)	Bacillales	Firmicutes	99
Seawater	*Micrococcus luteus* (HM146150)	Actinobacteridae	Actinobacteria	99
Seawater	Uncultured *Alteromonas* sp. *(HQ836412)*	Gammaproteobacteria	Proteobacteria	100
Seawater	*Roseobacter* sp. (GU826622)	Alphaproteobacteria	Proteobacteria	97
Seawater	Uncultured *Neptuniibacter* sp. (JN210787)	Gammaproteobacteria	Proteobacteria	99
Seawater	*Alteromonas* sp. (JN654450)	Gammaproteobacteria	Proteobacteria	99
Seawater	*Yeosuana aromativorans* (HM032797)	Flavobacteria	Bacteroidetes	97
Seawater	Uncultured bacterium clone (JN172581)	-	-	99
Seawater	*Pseudomonas* (DQ299933)	Gammaproteobacteria	Proteobacteria	99
Crude oil[a]	Uncultured Rhodobacteraceae bacterium (EU328076)	Alphaproteobacteria	Proteobacteria	98
Crude oil[a]	*Pannonibacter phragmitetus* (HM030756)	Alphaproteobacteria	Proteobacteria	100
Crude oil[a]	*Thalassospira* sp. (FJ662896)	Alphaproteobacteria	Proteobacteria	100
Crude oil[a]	*Pseudomonas* sp. (GU929556)	Gammaproteobacteria	Proteobacteria	99
Crude oil[a]	*Xanthobacter autotrophicus* (GU195173)	Alphaproteobacteria	Proteobacteria	100

[a]Isolates obtained from weathered crude oil MSM plates.

Table 3. The results identified hydrocarbonoclastic organisms similar to genera *Pseudomonas, Roseobacter, Alteromonas,* and *Yeosuana aromativorans* (Table 3).

Discussion

Marine oil spills off the Australian coast have ranged from major) (*Kirki*, approx. 17, 280 tonnes) to minor spills (*Sanko Harvest*, approx. 700 tonnes) [2]. South Australian oil spills include the *Esso Gippsland*, Port Stanvac (1982) releasing fuel oil into the Gulf St. Vincent; the *Era*, Port Bonython (1992) releasing some 300 tonnes of bunker fuel into the Spencer Gulf and the *Chanda*, Port Stanvac (1999) releasing some 230 tonnes of crude oil into the Gulf St. Vincent [2]. In all these cases, the degradation treatment strategy was the application of dispersants and natural degradation. This response was elected owing to weathering conditions and access to certain areas affected being inaccessible (Mangroves; *Era* spill). The fate of hydrocarbons in the aquatic environment has been well documented [11, 20, 26] including details on the influence of abiotic and biological factors. In addition, many biodegradation parameters have been studied including salinity, nutrients, temperature, and oxygen availability. Although such studies provide a comprehensive overview pertaining to the influence of these factors, they do not encompass the importance of the endogenous oil microbial community. This study has investigated the role of the native oil community in biodegradation of weathered crude hydrocarbons that persist in the marine environment long after the primary spill.

In the occurrence of an aquatic oil spill, the immediate process of weathering commences. The oil immediately goes through a variety of physical, chemical and biological changes [26]. It is these weathering processes that alter the composition and properties of the oil that consequently influence the response strategy. Those hydrocarbons of larger chain lengths (C_{20}–C_{40}) are hydrophobic solids difficult to degrade owing to their low aqueous solubility, bioavailability, and structure [25]. Therefore, high molecular mass compounds are frequent end-products, often termed recalcitrant compounds as they do not respond well to remediation treatments and are usually found in weathered crude oils [23]. The characteristics of weathered crude oil are shown by the gas chromatograms of the hydrocarbon source used in this study (Fig. 1A). The gas chromatogram analysis showed that a majority of fractions in the TPH profile were comprised of C_{30} to above C_{40} hydrocarbons.

The seawater sample used in this study was from the Gulf St. Vincent, South Australia, an area of pre-exposed hydrocarbon contamination. However, despite the history of oil spills in this region, the seawater alone had minimal (1.2% cultivable community; Table 1) hydrocarbon degraders, a possible result of oceanic movement and/or selective pressures. As such, water samples from this region, given their pre-exposure to hydrocarbons, provide a paradigm to the native microbial community's capacity to regenerate to a previously unexposed state. These studies enable us to investigate the role and function of indigenous oil communities when reproducing an oceanic oil spill. This study showed that the indigenous crude oil community was able to break down weathered crude oil in aquatic systems (either saline or sterile non-saline), significantly reducing the total petroleum concentration (Table 2).

As hydrocarbonoclastic organisms are so vital to the bioremediation of petroleum products, it makes oil microflora a valuable resource. Assessment of the seawater microbial community profile using DGGE analysis revealed a strong and diverse bacterial community, which increased in evenness over time. This increase in diversity and evenness in the microbial community shows the community adapted to the presence of the hydrocarbon over the course of the experiment. Identification of the key organisms that play a role in pollutant biodegradation is important for understanding, evaluating, and developing *in situ* bioremediation strategies [10]. Bacterial sequences isolated from the weathered crude oil presented an abundance of Alphaproteobacteria. However, the abundance of Alphaproteobacteria decreased with the addition of seawater. Sequence identification from the seawater microcosms showed the community shifted with an increase in Gammaproteobacteria, which is common in the event of a marine spill [12]. The bacterial sequences identified from the microcosms included species related to the genera *Alteromonas, Pseudomonas, Roseobacter,* and *Yeosuana aromativorans*. The occurance of these well-known hydrocarbon-degrading strains in hydrocarbon-contaminated enviroments is not uncommon [4, 6, 24] and had previously been thought to be associated with the environment where the oil was contaminated rather than also being present in the contaminating oil. This process of natural attenuation is essential and a primary way by which crude oil is eliminated from a contaminated site [24]. However, despite this rich natural community, most of the hydrocarbon-degrading organisms have been isolated and enriched from polluted environments including soil and marine [19]. This study has shown no enrichment methods were needed to isolate these hydrocarbon degraders, as the oil microflora was well established. However, when characterizing environmental samples for hydrocarbon degraders, the cell density is usually not high enough for direct isolation on selective plates and therefore requires enrichment procedures [21].

The data presented in this study clearly indicate that the native oil community has a highly influencing role in the removal of weathered crude oil from an aquatic environment. This is largely an unexplored reservoir of hydrocarbonoclastic microbes that could be examined for commercialization.

However, a limitation of identifying hydrocarbonoclastic organisms is the large number of unidentified sequences in the databases. Despite new technology advancements, traditional microbial techniques are still vital for isolation and characterization, as are their use in conjunction with molecular techniques for activity and identification. Future work in this area will focus upon the isolation of individual bacterial strains from the native oil community and examine their influence on a remediation event. Such information will greatly contribute to our current understanding of the complexities and diversity of the native weathered oil community.

In conclusion, the natural crude oil community is a valuable resource for bioremediation of pollutant environments. The hydrocarbonoclastic bacteria naturally present are able to adapt to different aquatic sources, and when exposed to a hydrocarbon source can promptly commence degradation. However, despite this and the substantial commercial application worldwide, previous studies continue to isolate and characterize hydrocarbon degraders from polluted environments including soil and marine, and disregard the hydrocarbon contaminant itself.

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