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Obligate Oil-Degrading Marine Bacteria

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Abstract

Over the past few years, a new and ecophysiolegically unusual group of marine hydrocarbon-degrading bacteria – the obligate hydrocarbonoclastic bacteria, or OHCB – has been recognized and shown to play a significant and global role in the biological removal of petroleum hydrocarbons from polluted marine waters. This recognition stimulated intensive efforts to characterize and exploit OHCB, so there have been a number of important developments during the last couple of years that we summarize here. Both *in situ* and experimental micro- and mesocosm studies have demonstrated that introduction of oil or oil constituents into seawater leads to successive blooms of a relatively limited number of indigenous marine bacterial genera - *Alcanivorax*, *Marinobacter*, *Thalassolituus*, *Cycloclasticus*, *Oleispira* and few others, the OHCB - which are present at low or undetectable levels prior to the polluting event. The types of OHCB that bloom depend on the latitude/temperature, salinity, redox and other prevailing physical-chemical factors. These blooms result in rapid degradation of many oil constituents, a process that can be accelerated further by supplementing with limiting nutrients, so have potential for the development of new biomitigation strategies to combat ecological damage resulting from oil spills in marine systems. The genome sequencing and functional genomic analysis of *Alcanivorax borkumensis*, the paradigm of OHCB and probably the most important OHCB globally, has provided significant insights into the genomic basis of the efficiency and versatility of its hydrocarbon utilization, the metabolic routes underlying its special hydrocarbon diet, and its ecological success. Other studies have revealed multiple food storage compounds – waxes and polyhydroxyalkanoates - formed when hydrocarbons are plentiful, and yielded a novel mutant that hyperproduces PHA and releases it into the medium. This result and others have revealed the potential of OHCB for multiple biotechnological applications that include not only oil pollution mitigation, but also biopolymer production and biocatalysis.

Introduction

Hydrocarbons and their derivatives, including solid, liquid and gaseous fossil carbon deposits, but also compounds of biological origin, such as lipids and fatty acids from plants, animals and microbes, and the products of their conversion in anoxic zones, are ubiquitous in the biosphere, though highly heterogeneous in type and concentration, and in time and space. Given the high carbon content available for biomass production, and the high energy content of such highly reduced compounds, it is hardly surprising that many microbes have evolved or acquired the ability to utilize hydrocarbons as sources of carbon and energy. Almost a century has passed since the first hydrocarbon-degrading bacteria were isolated and described, and the most recent list includes almost 200 bacterial, cyanobacterial, algal and fungal genera, representing more than 500 species and strains [1, *2].

Despite the ubiquity of hydrocarbons in marine systems, originating from natural seeps of oil and natural gas deposits, marine oil transport accidents and deliberate discharges, and from biomass and biological processes, true marine hydrocarbon-degrading microbes were only discovered relatively recently. Interestingly, and in contrast to terrestrial hydrocarbon degraders, which tend to be metabolically versatile and utilize a large range of organic substrates, their marine counterparts are mostly highly specialized obligate hydrocarbon utilizers, the so-called marine “obligate hydrocarbonoclastic [from Greek *klas'ti.cus*. M. L. part. adj. clastic, breaking down] bacteria”. Recent work has revealed that the OHCB play a significant and global role in the natural cleansing of oil-polluted marine systems. We review here latest results pertaining to the biogeography, ecophysiology, genomics and potential for biotechnological applications of OHCB.

Taxonomy of Obligate Hydrocarbonoclastic Bacteria

Of the diverse range of oil degrading bacteria isolated to date, less than a quarter have been obtained from marine sources, and only strains from 19 genera of *Eubacteria* could by their obligate requirement for NaCl-containing growth media be characterized as indigenous marine organisms (Figure 1). Only two NaCl-dependent hydrocarbon-degrading strains of the *Firmicutes* and *Bacteroidetes* phyla have so far been isolated, namely the alkane degrader *Planomicrobium alkanoclasticum* MAE2 [3] and the PAH degrader *Yeosuana aromativorans* GW1-1 [4], respectively. The remaining aerobic marine hydrocarbon-degrading isolates are all affiliated with either the alpha- or the gamma-*Proteobacteria* subclasses, mostly the latter. All of these bacteria exhibit a “BIOLOG anomaly”: that is, growth occurs on only two of the 95 substrates of the BIOLOG® system, namely on Tween 40 and Tween 80, substrates that contain long-chain alkyl moieties.

The highly specialized substrate specificity - obligate hydrocarbon utilization - seems to be more characteristic for marine hydrocarbon degrading bacteria than soil bacteria: thus far only three paraffin-degrading bacteria possessing similar narrow substrate profiles have been isolated from terrestrial environments [5,6].

Since the description of the first OHCB, *Alcanivorax borkumensis* [7,8], four further genera of OHCB, represented by seven validly published species, have been reported (Figure 1). The genera *Alcanivorax* and *Cycloclasticus* include both OHCB, like *A. borkumensis*, *A. jadensis* [8, 9], *A. dieselolei* [10], *C. pugetii* [11], and *C. oligotrophus* [12], and more nutritionally versatile species with less restricted substrate profiles, like *A. venustensis* and *C. spirillensus* [9,13]. The three other genera of the OHCB group, despite being represented by numerous members, are monophyletic and represented by the three type species *Oleiphilus messinensis* [14], *Oleispira antarctica* [15] and *Thalassolituus oleivorans* [16].

Biogeography of OHCB

The physiology, ecology and biogeography of OHCB have been discussed in a number of earlier reviews, which were however necessarily restricted mainly to *Alcanivorax* and *Cycloclasticus* [*2, 8, 17-20]. The current situation (end of 2006) is that more than 250 *Alcanivorax*-affiliated bacteria have been isolated or detected as 16S rRNA gene sequences in all types of marine environment: surface water, shallow and deep-sea water bodies, sediments ([*2, 21]), hydrothermal vents and mud volcanoes, ridge flank crustal fluids and gray whale carcass (s. GenBank Acc. Nrs. AB166993, AY345573; [22-24]), in corals, sponges and aquaculture-poisoning dinoflagellates (s. GenBank Acc. Nrs. DQ889910, AF489287; [25, 26]). *Alcanivorax*-like organisms have also been detected in a few terrestrial environments whose characteristics share relevant properties (salinity, presence of hydrocarbons) with marine ecosystems, e.g. selenium-contaminated hypersaline evaporation pond in California [27], saline subsurface waters in Africa and Australia (GenBank Acc. Nr. DQ337077; [28]), hydrocarbon-polluted saline soil ([29]) and various geothermal areas in Italy and USA (Yellowstone) [30, 31] (Figure 2). This ubiquity of *A. borkumensis* is presumably due to its capacity to grow on many saturated petroleum fraction constituents and on biogenic hydrocarbons: straight-chain and branched alkanes, isoprenoids, and long-side-chain alkyl compounds, including alkylmonocycloalkanes, alkylbenzenes, and organic alkyl- sulfuric compounds [32, 33]. *A. borkumensis* associated with marine invertebrates seems to reflect a special ecological niche containing readily accessible hydrocarbons produced by the animal partners. Interestingly, although *Alcanivorax*-related 16S rRNA gene sequences have been retrieved from microbial communities inhabiting cold polar areas, the organism itself has so

far only been isolated from more temperate lower latitudes.

Two other OHCB, *T. oleivorans* and *Cycloclasticus* spp., are also widely distributed, though mostly found so far in the Northern hemisphere of the planet (Figure 2), possibly due to sampling bias. The GenBank and RDP databases currently contain 16S rRNA gene sequences of 59 *Thalassolituus*-like bacteria originating from microbial communities inhabiting both marine (Baltic, Barents, Mediterranean, North, Okhotsk and South China seas, and the Atlantic, Pacific and Polar oceans) and terrestrial environments (subsurface caves and ground waters) [34, 35]. In contrast, all of the 38 sequences in the databases assigned to the genus *Cycloclasticus* were retrieved exclusively from marine microbial communities, or PAH-supplemented enrichments thereof [10,12, 36, 37].

In contrast to the cosmopolitan OHCBs discussed above, distribution of the psychrophilic OHCB *Oleispira antarctica* (55 sequences until now) is thus far limited to the colder waters found at high latitudes [38, 39]: the least cold sites at which *O. antarctica* has been found are Cape Cod (MA, USA, GenBank Acc.Nr.AM117931) 42°04'26"N, 70°12'19"W and South Tasmania [40].

The most enigmatic marine OHCB, *Oleiphilus messinensis*, initially isolated from the sediments (harbor of Messina Italy, [14], seems to thrive elsewhere as a sponge symbiont, since *Oleiphilus*-like 16S rRNA gene fragments have been recovered from bryozoan and dictyoceratid sponges sampled in North Atlantic and equatorial Pacific oceans [41, 42].

Genomic basis of OHCB ecophysiology

Marine OHCB occupy a special trophic niche among marine heterotrophic bacteria participating in the global carbon cycle, since they mediate degradation of chemically stable saturated and aromatic hydrocarbon species that are not substrates for most bacteria. The ecophysiology of OHCB has not been studied extensively since, although their isolation is now rather simple, their discovery was relatively recent and many laboratories have experienced difficulty in maintaining them in pure culture in a metabolically active form over long periods of time. However, a few studies have revealed unusual and interesting features of the marine OHCB lifestyle. Organisms analysed so far exhibit features typical of oligotrophic bacteria [43,**44]. The most detailed studies, carried out with *C. oligotrophus*, have shown that the cytoplasm of this small bacterium is very dilute, with a dry mass per cell 7-8 times lower than that of *E. coli*, but a DNA content of up to 14% dry weight, which contrasts the value of 2% for *E. coli* [13, 43]. Remarkably, the outer cellular membrane is enriched for a wide range of transport systems for the capture of nutrients and diverse oligo-elements from the generally nutrient-poor marine

environment [43,**44]. The affinity of *C. oligotrophus* cells for toluene is the highest microbial cell:substrate affinity reported so far and is sufficient for bacterial growth in seawater containing hydrocarbons at exceptionally low concentrations [43].

Genomic analysis of the *A. borkumensis* strain SK2 has revealed a large repertoire of genetic determinants for the uptake of mineral nutrients limiting in marine environments, particularly following a sudden input of oil, which leads to severe imbalances in C/N and C/P ratios. *A. borkumensis* encodes a wide range of transport proteins, among them determinants for about fifty permeases, about half of which are high affinity ABC-transport systems [**44]. The genome encodes two clusters of genes for active nitrate uptake and reduction (*nrtCB-nasDTS* and *narKGHJI*), and determinants for three high-affinity ammonium transporter systems (*amt*). Phosphate uptake is mediated by a high-affinity ABC-type system, composed of the *phoU-psiBACS* and *phoBR* gene products, and, under eutrophic conditions (>20mM Pi), by a low-affinity Pit transporter system. *Alcanivorax* specifies the uptake of a number important oligoelements, like magnesium, molybdate, zinc and cobalt, through *mgtE*, *modABC*, *znuAB*-encoded systems and a CorA-like MIT family protein. This battery of genetic determinants for scavenging functions enables *A. borkumensis* SK2 to efficiently exploit its alkane-catabolic functions in response to a sudden appearance of hydrocarbons, and adapt to carbon:nutrient imbalances that occur for example after an oil spill, and may explain the competitive advantage *Alcanivorax* enjoys in such circumstances.

No genes for either passive or active carbohydrate transporters, that are usually present in other bacteria, were identified in the genome, which is consistent with the “BIOLOG anomaly”, and inability to use monomeric sugars as growth substrates [**44, 45].

A. borkumensis strain SK2 degrades straight-chain alkanes up to C₃₂ in length, long-chain isoprenoids, phytane and pristane, and alkyl-aromatic hydrocarbons [32, 33]. Although growth on isoprenoids typically involves a long lag-phase, doubling times of exponentially-growing cultures are similar on hexadecane (0.115±0.03 h⁻¹) and pristane (0.106±0.016 h⁻¹). The SK2 genome specifies multiple systems for hydrocarbon catabolism, namely two alkane hydroxylase systems AlkB1 and AlkB2 and three P450 cytochromes [**44, 46, *47, 48, 49]. Both alkane hydroxylase systems are located close to the origin of replication of the chromosome, which provides a high gene dosage and presumably high expression levels of these catabolic systems. Proteomic profiling suggested that both AlkB systems and all three cytochromes P450(a-c) participate in catabolism of saturated hydrocarbons [*50]. Quantitative real-time transcriptional analysis showed that the P450(b) and P450(c) genes, which encode identical polypeptide sequences, were

expressed only in the presence of alkane, whereas P450(a) was also expressed in cells growing on pyruvate. Similar expression profiles of the *alkB1*, *alkB2* and cytochromes P450(a,b,c) genes were observed in exponential cultures growing on *n*-tetradecane. Interestingly, in phytane-grown cells, strong induction of the cytochrome P450(a) gene was found, but no transcription of *alkB1*. Such a differential expression of these two genes may be useful in the application of *A. borkumensis* gene transcription profiling as a possible bio-indicator of oil pollution [**44].

The *alkSB1GHJ* gene clusters are found in a number of bacteria, e.g. in the genome-sequenced obligate marine hydrocarbon-degrading *Marinobacter aquaeolei* (strain VT8) and the ubiquitous alpha-proteobacterium *Oceanocaulis alexandrii* (strain HTCC2263) (Fig. 3A). Comparative sequence analysis of *alkS/alkB1* genomic regions revealed that the genetic organization in these bacteria and *A. borkumensis* is more similar than that found in soil pseudomonads [51]). As it has been shown previously for a number of alkane-degrading microorganisms, the entire *alkSB1GHJ* gene cluster is a prominent region of alien origin, typically characterized by a significantly lower G+C content than the rest of the genome [2004]. This is also the case for these three marine bacteria: the G+C content of *alkSB1GHJ* in *O. alexandrii* is 7.8% lower than the average, in *A. borkumensis* – 6.8%, in *M. aquaeolei* – 7.4% and 4.1% (two distinct *alkB* clusters). The bracketing of the *alkSB1GHJ* clusters of *M. aquaeolei* and *O. alexandrii* with putative transposase genes (Figure 3(A)) is consistent with an earlier observation that gene clusters for alkane degradation can be transferred among bacteria via mobile genetic elements [51].

A similarly ubiquitous genetic organization also exists for determinants specifying degradation of aromatic hydrocarbons. Analysis of the organization of gene clusters in *Cycloclasticus spp.* for the degradation of (poly)aromatic hydrocarbons revealed a cluster of six open reading frames *xyiXMKG1C2* that specifies utilization of all three forms of xylene [52] and that exhibits similar organization to the catabolic determinants of the PAH-degrading freshwater beta-proteobacteria, *Burkholderia xenovorans* LB400 and *Polaromonas naphthalenivorans* CJ2. Sequence analysis of a 10.5-kb DNA fragment from *Cycloclasticus sp.* A5 revealed a cluster of *phn* genes specifying degradation of naphthalene, methylnaphthalene, phenanthrene and dibenzothiophene. The *phnA1*, *phnA2*, *phnA3*, and *phnA4* genes, coding for the α - and β -subunits of an iron-sulfur protein, a ferredoxin, and a ferredoxin reductase, respectively, encode the initial enzyme of the pathway, PAH dioxygenase [53]. As can be seen in Fig. 3(B), the genetic organization of the *phn* gene cluster of the gamma-proteobacterial *Cycloclasticus* is similar to that found in several alpha-proteobacteria. The *phn* gene cassette of *Novosphingobium aromaticivorans* F199 is located on a large plasmid, pNL1 [54]. Interestingly, the *pdxA* gene, which specifies an enzyme involved in the

biosynthesis of coenzyme PLP, is clustered and co-oriented with *pbnA1b*, seemingly a characteristic feature of *pbn*-like cassettes (Figure 3(B)). The broad PAH substrate range characteristic of *Cycloclasticus* may reflect the existence of multiple dioxygenase determinants in the genome of this bacterium, analogous to the situation for alkane monooxygenases found in *Alcanivorax*. Interestingly, a third putative dioxygenase, homologous to the initial enzyme of the pathway for the degradation of 2,4-dichlorophenoxyacetic acid (2,4-D), has been detected in *C. oligotrophus* [52]. This indicates that *Cycloclasticus* spp. may degrade not only diverse (poly)aromatic hydrocarbons but also chlorinated derivatives thereof, and is consistent of their ubiquity in marine environments.

Marine OHCB in oil-degrading communities

A number of studies have shown that an influx of oil in a marine site causes population densities of HCB to transiently increase up to 90% of the total microbial community. Aliphatic hydrocarbon-degraders, in particular *Alcanivorax*, are the first to bloom and are succeeded by microbes, particularly *Cycloclasticus* spp., specialized for the compounds more difficult to degrade that remain [55, *56, see also reviews *2, 17, 20]. Such studies have been essentially replicated in experimental systems, involving either unpolluted sea water subsequently spiked with oil, or samples from polluted sites, and have confirmed the pivotal role of OHCBs in marine microbial communities confronted with oil hydrocarbons. For example, Yakimov and co-authors [57] studied community population shifts in microcosms of superficial sediments from a chronically polluted area of the Milazzo Harbour oil refinery, Sicily, that were experimentally spiked with oil, tetradecane or naphthalene, with or without supplementation with mineral nutrients. The initial community, composed mostly of vibrios, low-GC Gram-positive bacteria, *Arcobacter* spp. and few crenarchaea, became dominated by HCB after addition of hydrocarbons: whereas *Alcanivorax* spp. dominated the OHCB bloom in crude oil-spiked microcosms, *Thalassolituus* spp. dominated tetradecane-spiked microcosms, and *Neptunomonas*-like microbes dominated naphthalene-spiked microcosms. Similar results indicating the central role of HCB, and *Alcanivorax* spp. in particular, were obtained in a mesocosm study involving a 14000 l bioreactor [58]. Microcosms of Thames salt marsh water from a site close to an oil refinery that were experimentally spiked with crude oil or oil constituents showed blooms of *Thalassolituus* spp. and *Roseobacter* spp., when spiked with crude oil, *Alcanivorax* spp., when spiked with the branched aliphatic hydrocarbon pristane, and *Cycloclasticus* spp., when spiked with PAHs [34]). A complementary study to analyze the effects of temperature and added nutrients revealed that *Alcanivorax* only appeared when nutrients were added, and that organisms affiliated to the genus *Oleispira* (first isolated as a cold-adapted OHCB

from crude-oil enrichments of Antarctic seawater [15], bloomed in microcosms maintained at 4°C [59]. *Oleispira* was also shown to be present in oil-degrading microbial communities in other microcosm studies performed at low temperatures. Brakstad and Bonaunet [60] identified *Oleispira* spp. in such a study, in addition to a number of species of gamma-*Proteobacteria* (e.g., *Psychromonas* spp.) and *Bacteroidetes*, and Gerdes et al. [39] emphasized the importance *Pseudomonas* spp., *Shewanella* spp. and *Marinobacter* spp. in oil-degrading communities of Arctic sea ice. *Marinobacter* spp., together with *Psychrobacter*, *Pseudoalteromonas* and *Shewanella*, were identified in an oil-degrading community established in Arctic sea ice [61].

Though revealing, such microcosm studies as closed systems are reductionist and artificial, since they lack the complexity and diversity and dynamics of natural inputs (e.g. mineral nitrogen and phosphorous), exports (e.g. excreted cellular metabolites) and predator grazing. Moreover, some studies were restricted in their community analysis to fingerprinting and clone library analysis of 16S rRNA genes, which are unable to establish causality between an organism and its physiology/environmental role. For instance, numerous isolates of *Thalassolituus oleivorans* obtained from the Polar coastal area of Russia, from seawater samples near an off-shore oil drilling platform (Sakhalin Peninsula), from the Mediterranean Sea, and from the North Sea, are identical in terms of their SSU rRNA gene sequences but exhibit very distinct substrate preferences and temperature requirements [62]. It is therefore not evident which metabolic capacity is represented by a particular rRNA gene sequence, especially considering the wide distribution among marine organisms of relatively conserved gene cassettes for hydrocarbon degradation on mobile elements. Despite these qualifications about experimental approaches, it is clear that oil hydrocarbon degradation in marine systems is carried out by microorganisms belonging to a relatively small group of genera, and that there are certain important differences in the compositions of oil-degrading communities at high and low latitudes that need to be considered when developing potential mitigation strategies to combat oil pollution in marine systems.

Potential biotechnological applications

The capacity of marine hydrocarbon-degrading microorganisms to efficiently degrade hydrocarbons and their potential use for mitigation of oil spills has been discussed elsewhere [*2, 18]. It has since become apparent, however, that such bacteria may also have the potential to be applied in other contexts. A recent functional genomics study of *A. borkumensis* SK2 revealed that inactivation of a “TesB”-like hydroxyacyl-coenzyme A-specific thioesterase leads to hyperproduction and extracellular localization of polyhydroxyalkanoates (PHA) [*63], even

though the normal food storage compounds found in this strain and other OHCB (*Marinobacter* and *Thalassolituus*) are triacylglycerols and wax esters [64]. The *tesB*-like mutation results in the channelling of CoA-activated hydroxylated fatty acids, the cellular intermediates of alkane degradation, almost exclusively towards PHA formation. The release of large quantities of PHA by mutant *Alcanivorax* cells allows recovery of PHA from the culture medium without a costly extraction of PHA from intracellular granules by environmentally-problematic solvents, and makes the mutant a potential *Alcanivorax* cell factory for biopolymer production [64, 65].

OHCB are both relatively recent discoveries, and have a novel physiology, so might be expected to have enzyme repertoires that are so far unprospected and potentially interesting for biocatalysis, the enzymatic biosynthesis of fine chemicals and added value compounds. A recent study of a metagenome expression library of a crude-oil enrichment of the seawater-brine interface of the Urania hypersaline anoxic basin (Eastern Mediterranean Sea) resulted in retrieval of novel enzymes [66]. Functional screening of the library resulted in the identification of five groups of carboxylesterases. The most abundant group, “Oil2”-type polypeptides (GenBank Acc. Nr. AJ811965), were affiliated with *Marinobacter* alpha-beta fold hydrolases; a second group consisted of the “O.02” carboxylesterase (AJ811965), which is highly similar to that of *A. borkumensis* SK2; a third (AJ811969) was the “O.23” carboxylhydrolase, possessing the carboxylhydrolase B domain and distantly related (ca. 50% protein sequence similarity) to the deduced enzyme from *Bacillus niacini*. Two other polypeptides were not affiliated to any known esterase: the “O.21” enzyme (AJ811968) exhibited low homology (below 25 % peptide sequence similarity) to conserved hypothetical proteins from xantomonades, and the “O.16” protein (AJ811967) had no phylogenetic affiliations whatsoever. All retrieved enzymes were characterised biochemically and exhibited good potential for biosynthetic applications, i.e. ability to function in polar organic solvents and to resolve chiral mixtures of a number of important drug precursors. The most peculiar protein, “O.16”, demonstrated the highest enantioselectivity ever reported for the ester of the important chiral synthon solketal ($E: 126[S]; 98\%ee$). This enzyme, from an as yet unknown hydrocarbon-degrader, contains three catalytic serine residues in two domains exhibiting distinct activities - thioesterase and carboxylesterase - and a unique adaptive structure: function characteristic that involves radical changes in its tertiary/quarternary structure in response to changes in environmental physical-chemical conditions.

Conclusions and perspectives

A significant input to our knowledge on the genomics of HCB is expected to result from the genomic analysis of another hydrocarbon degrading marine gamma-*Proteobacterium*, *Marinobacter aquaolei* VT-8, also known in the literature of the 1970-80s as *Pseudomonas nautical* and synonymous with *M. hydrocarbonoclasticus*. The sequencing of VT-8 by the Joint Genome Institute (http://genome.jgi-psf.org/mic_home.html) is already finished and has yielded a bacterial chromosome as a single contig, and two megaplasmids each of about 200 kbp (available online at the GenBank website as RefSeq. NC_008738, NC_008739 and NC_008740 ([http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genomeprj&cmd=search&term=txid351348\[orgn\]](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genomeprj&cmd=search&term=txid351348[orgn]))).

Marinobacter species are important marine hydrocarbon degraders that are metabolically more versatile (e.g. in their ability to denitrify) than *Alcanivorax*. The reason for this greater versatility is certainly to be found in its approx. 1500 additional genes that *Alcanivorax* lacks, so the *in silico* analysis of its genome, and complementary functional studies, will reveal the genomic basis of the niche-specific features of *Marinobacter*. Another gamma-*Proteobacterium* belonging to the true OHCBs whose genome sequence will soon become available is the psychrophilic alkane-degrader *Oleispira antarctica* RB-8^T. Comparative genomics and metabolic network modelling of these and other marine hydrocarbon-degrading bacteria will indicate the core genetic determinants of an efficient hydrocarbon-degrader and provide a genomic framework for synthetic biology approaches to generate an OHCB with a “minimal” streamlined genome.

Critical to such studies are functional genomic analyses of OHCBs needed to yield *inter alia* critical understanding of OHCB responses to the varying stresses experienced in the marine environment (osmotic, solute, temperature, pressure, UV-irradiation, etc.), which is of crucial importance for development of effective strategies for biomitigation of oil spills. Genetic tools that have been developed for the study of other gamma-*Proteobacteria*, though not as developed as those for *E. coli*, work well for the study and manipulation of OHCB and enable use of classical genetic approaches to establish genotype:phenotype links and functional causalities.

The development of useful biomitigation intervention strategies will also require new insights from the modelling of natural and experimental marine microbial networks, in terms of process rates, metabolite/intermediate concentrations and fluxes, and the behaviour of individual functional members of such networks, in a manner nicely exemplified by a recent case study on anaerobic biodegradation of organic matter by a complex microbial community [67]. For such modelling, statistically robust data must be obtained from real-time measurements in large-scale simulations. Moreover, new knowledge is urgently needed on the critically important activities

and roles of predators and grazers (viral, prokaryotic, eukaryotic) on the composition, population dynamics and ecophysiological functioning of marine oil-degrading communities, and the role of lysogenic phages in their functioning, adaptation and evolution.

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Figure Legends

Figure 1. Phylogenetic affiliations of marine hydrocarbon-degrading bacteria. The obligate hydrocarbonoclastic bacteria are highlighted in boldface. Strains obtained in enrichment cultures, but not isolated in pure culture, are indicated by asterisks. The tree, based on 1360 nucleotide positions, was constructed by the neighbour-joining method and nucleotide substitution rates were calculated by using Kimura's two-parameter model. The tree was out-grouped and rooted with 16S rDNA sequences of *Thermotoga petrophila* (AB027016) and *Deinococcus radiodurans* (AF289089), respectively.

Figure 2. Geographic distribution of isolates or retrieved 16S rRNA gene sequences of marine obligate hydrocarbonoclastic gamma-proteobacteria

Figure 3. Ubiquity of gene clusters for the degradation of (A) aliphatic and (B) (poly)aromatic fractions of oil. (A) Organization of genes homologous to the *A. borkumensis* *alk* gene cluster in hydrocarbon-degrading marine proteobacteria. Homologous genes are highlighted by shaded areas: sequences predicted to code for LuxR-type transcriptional activators of the alkane genes, AlkS, are marked in green, genes for the alkane degradation pathway are indicated in blue, and transposase-related sequences are shown in red. Percentages of protein identity/similarity of polypeptides from *A. borkumensis* with those of *M. aquaeolei* and *O. alexandrii* are shown. Gene designations: *alkB1*, alkane monooxygenase; *alkG*, rubredoxin; *alkJ*, alcohol dehydrogenase; *alkH*, aldehyde dehydrogenase. **(B)** Organization of gene clusters of PAH degradation pathways of *Cycloclasticus* sp. A5 and PAH-degrading alpha-proteobacteria. *pdxA* genes predicted to code for the pyridoxal phosphate biosynthesis enzyme are coloured in green, PAH degradation genes are coloured in blue. Percentages of protein identity/similarity of polypeptides from *Cycloclasticus* sp. A5 with those of the PAH-degrading alpha-proteobacteria are shown. Gene designations: *pbnA1*, iron-sulfur protein (ISP) α subunit of PAH dioxygenase; *pbnA2*, ISP β subunit of PAH dioxygenase; *pbnA3*, Rieske-type [2Fe-2S] ferredoxin, *pbnA4*, NADH-ferredoxin oxidoreductase; *pbnC*, extradiol [3,4-dihydroxyphenanthrene] dioxygenase; *pbnD*, 2-hydroxy-2H-benzo[*b*]chromene-2-carboxylate isomerase.