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Obligate oil-degrading marine bacteria

Michail M Yakimov[1], Kenneth N Timmis[2,3] and Peter N Golyshin[2,3]

Over the past few years, a new and ecophysiologically unusual group of marine hydrocarbon-degrading bacteria – the obligate hydrocarbonoclastic bacteria (OHCB) – has been recognized and shown to play a significant role in the biological removal of petroleum hydrocarbons from polluted marine waters. The introduction of oil or oil constituents into seawater leads to successive blooms of a relatively limited number of indigenous marine bacterial genera — *Alcanivorax*, *Marinobacter*, *Thallassolituus*, *Cycloclasticus*, *Oleispira* and a few others (the OHCB) — which are present at low or undetectable levels before 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 the rapid degradation of many oil constituents, a process that can be accelerated further by supplementation with limiting nutrients. Genome sequencing and functional genomic analysis of *Alcanivorax borkumensis*, the paradigm of OHCB, 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. These and other studies have revealed the potential of OHCB for multiple biotechnological applications that include not only oil pollution mitigation, but also biopolymer production and biocatalysis.

Addresses
[1] Istituto per l'Ambiente Marino Costiero, CNR, Messina 98122, Italy
[2] Division of Microbiology, HZI – Helmholtz Centre for Infection Research, 38124 Braunschweig, Germany
[3] Institute of Microbiology, Technical University of Braunschweig, Biozentrum, 38106 Braunschweig, Germany

Corresponding author: Timmis, Kenneth N (kti@helmholtz-hzi.de)

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Introduction

Hydrocarbons and their derivatives, including solid, liquid and gaseous fossil carbon deposits, 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. 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•].

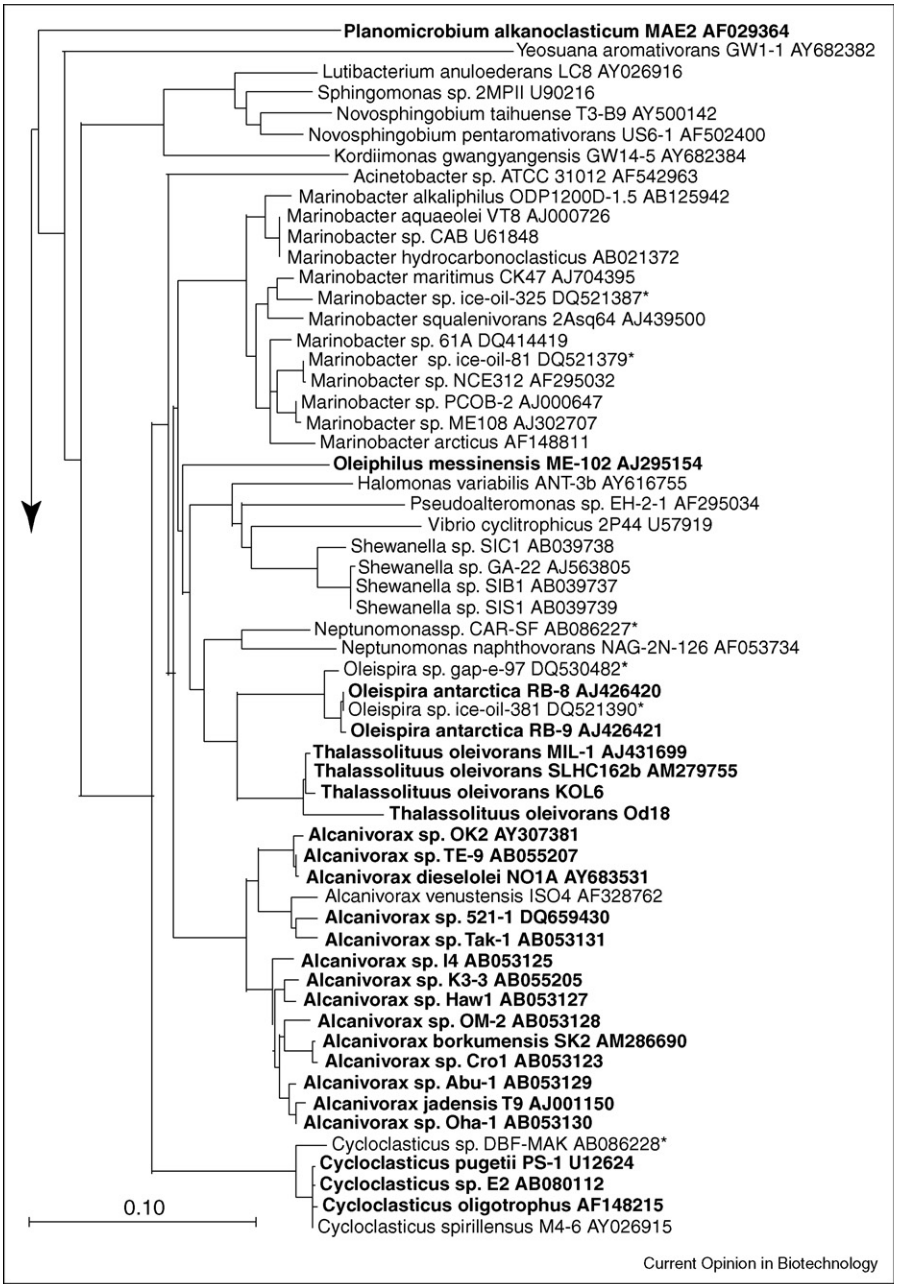
Despite the ubiquity of hydrocarbons in marine systems — originating from natural seeps of oil and natural gas deposits, marine oil transport accidents and deliberate discharges, and from biomass and biological processes — true marine hydrocarbon-degrading microbes were only discovered relatively recently. 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 bacteria' (OHCB). 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 OHCB

Of the diverse range of oil-degrading bacteria isolated to date, less than a quarter have been obtained from marine sources. Strains from only 19 genera of *Eubacteria* could — through 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 polyhydroxyalkanoate (PAH) degrader *Yeosuana aromativorans* GW1-1 [4], respectively. The remaining aerobic marine hydrocarbon-degrading isolates are all affiliated with either the α- or the γ-*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. This 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,

Figure 1



Phylogenetic affiliations of marine hydrocarbon-degrading bacteria. The obligate hydrocarbonoclatic 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.

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

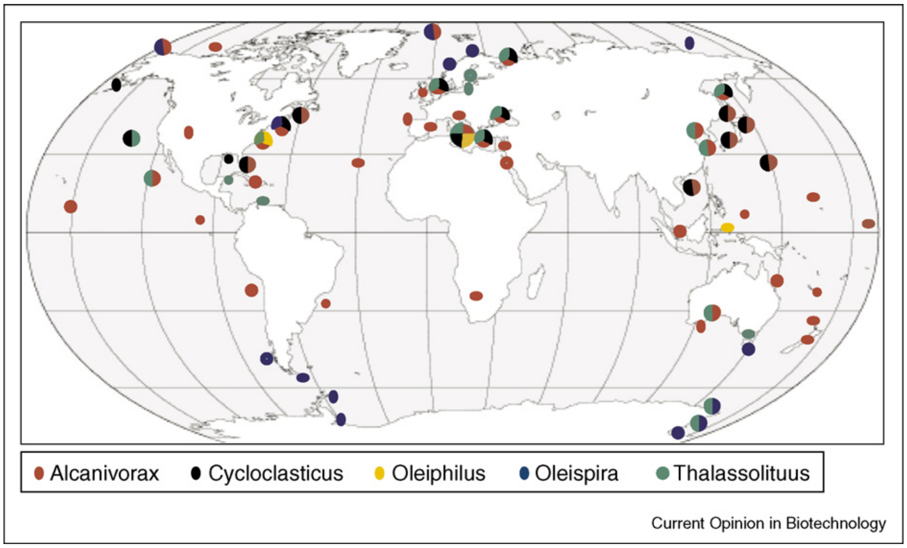
The physiology, ecology and biogeography of OHCB have been discussed in several earlier reviews; however, these were necessarily restricted mainly to *Alcanivorax* and *Cycloclasticus* [2•,8,17–20]. The current situation (end of 2006) is that more than 250 *Alcanivorax*-affiliated bacteria have been isolated or detected as 16S rRNA gene sequences in all types of marine environments: surface water, shallow and deep sea water bodies, sediments ([2•,21]), hydrothermal vents and mud volcanoes, ridge flank crustal fluids and grey whale carcass (GenBank accession numbers AB166993, AY345573; [22–24]), in corals, sponges and aquaculture-poisoning dinoflagellates (GenBank DQ889910, AF489287; [25,26]). *Alcanivorax*-like organisms have also been detected in a few terrestrial environments that share relevant properties (salinity, presence of hydrocarbons) with marine ecosystems; for example, a selenium-contaminated hypersaline evaporation pond in California [27], saline subsurface waters in Africa and Australia (GenBank 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* presumably results from 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 (e.g. alkylmonocycloalkanes, alkylbenzenes and organic alkyl-sulfuric compounds) [32] (MM Yakimov *et al*, unpublished). *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, although so far they have mostly been found in the Northern hemisphere (Figure 2), possibly due to sampling bias. The GenBank and RDP (Ribosomal Databse Project) 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) [33,34]. By 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 [10,12,35,36].

In contrast to the cosmopolitan OHCBs discussed above, distribution of the psychrophilic OHCB *Oleispira antarctica* (55 sequences to date) is thus far limited to the colder waters found at high latitudes [37,38]: the least cold sites at which *O. antarctica* has been found are Cape Cod (MA, USA; GenBank AM117931), 42°04′26″N, 70°12′19″W and South Tasmania [39].

The most enigmatic marine OHCB, *Oleiphilus messinensis*, initially isolated from harbor sediments (Messina Italy [14]) seems to thrive elsewhere as a sponge symbiont, as *Oleiphilus*-like 16S rRNA gene fragments have been recovered from bryozoan and dictyoceratid sponges sampled in North Atlantic and equatorial Pacific oceans [40,41].

Figure 2



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

Genomic basis of OHCB ecophysiology

Marine OHCB occupy a special trophic niche among marine heterotrophic bacteria participating in the global carbon cycle, as 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 because, although their isolation is now rather simple, their discovery was relatively recent and many laboratories have experienced difficulty in maintaining these organisms 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 [42,43••]. 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 *Escherichia coli*, but a DNA content of up to 14% dry weight, which contrasts with 2% for *E. coli* [13,42]. 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 [42,43••]. 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 [42].

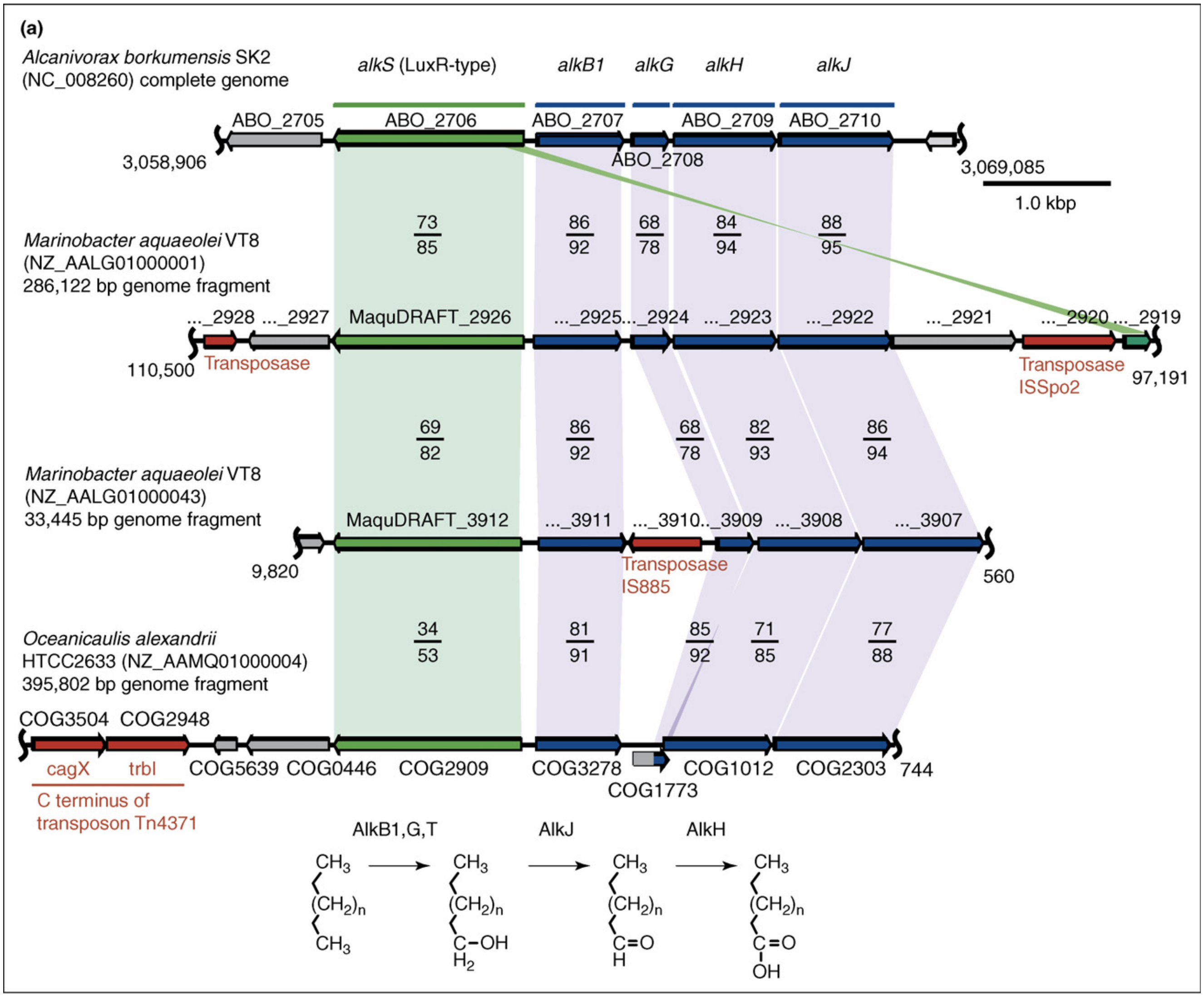
Genomic analysis of the *A. borkumensis* strain SK2 has revealed a large repertoire of genetic determinants for the uptake of mineral nutrients that are limiting in marine environments, particularly following a sudden input of oil, which leads to severe imbalances in carbon/nitrogen and carbon/phosphorus ratios. *A. borkumensis* encodes a wide range of transport proteins, among them determinants for about 50 permeases, about half of which are high-affinity ABC transport systems [43••]. 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–pstBACS* and *phoBR* gene products and, under eutrophic conditions (>20mM Pi), by a low-affinity Pit transporter system. *Alcanivorax* specifies the uptake of a number of important oligo-elements, like magnesium, molybdate, zinc and cobalt, through *mgtE*, *mod*ABC and *znuAB*-encoded systems and a CorA-like MIT (metal ion transport) 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 to adapt to carbon:nutrient imbalances that occur (e.g. after an oil spill). These functions could explain the competitive advantage *Alcanivorax* enjoys in such circumstances. No genes for either passive or active carbohydrate transporters, which are usually present in other bacteria, were identified in the genome. This observation is consistent with the BIOLOG anomaly and the inability to use monomeric sugars as growth substrates [43••,44].

A. borkumensis strain SK2 degrades straight-chain alkanes up to C_{32} in length, long-chain isoprenoids, phytane and pristine, and alkyl-aromatic hydrocarbons [32] (MM Yakimov *et al*, unpublished). Although growth on isoprenoids typically involves a long lag-phase, doubling times of exponentially growing cultures are similar on hexadecane ($0.115 \pm 0.03\ h^{-1}$) and pristane ($0.106 \pm 0.016\ h^{-1}$). The SK2 genome specifies multiple systems for hydrocarbon catabolism, namely two alkane hydroxylase systems AlkB1 and AlkB2 and three P450 cytochromes [43••,45,46•,47,48]. 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 [49•]. 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 alkanes, whereas P450(a) was also expressed in cells growing on pyruvate. Similar expression profiles of the *alk*B1, *alk*B2 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 *alk*B1. Such a differential expression of these two genes could be useful in the application of *A. borkumensis* gene transcription profiling as a possible bio-indicator of oil pollution [43••].

The *alk*SB1GHJ gene clusters are found in several bacteria (e.g. in the genome-sequenced obligate marine hydrocarbon-degrading *Marinobacter aquaeolei* strain VT8 and the ubiquitous α-proteobacterium *Oceanocaulis alexandrii* strain HTCC2263; Figure 3a). Comparative sequence analysis of *alk*S/*alk*B1 genomic regions revealed the genetic organization in these bacteria and *A. borkumensis* to be more similar than that found in soil pseudomonads [50]). As shown previously for several alkane-degrading microorganisms, the entire *alk*SB1GHJ gene cluster is a prominent region of alien origin, typically characterized by a significantly lower G+C content than the rest of the genome. This is also the case for these three marine bacteria: the G+C content of *alk*SB1GHJ in *O. alexandrii* is 7.8% lower than the average, in *A. borkumensis* it is 6.8% lower, and in *M. aquaeolei* 7.4% and 4.1% lower (two distinct *alkB* clusters). The bracketing of the *alk*SB1GHJ clusters of *M. aquaeolei* and *O. alexandrii* with putative transposase genes (Figure 3a) is consistent with an earlier observation that gene clusters for alkane degradation can be transferred among bacteria via mobile genetic elements [50].

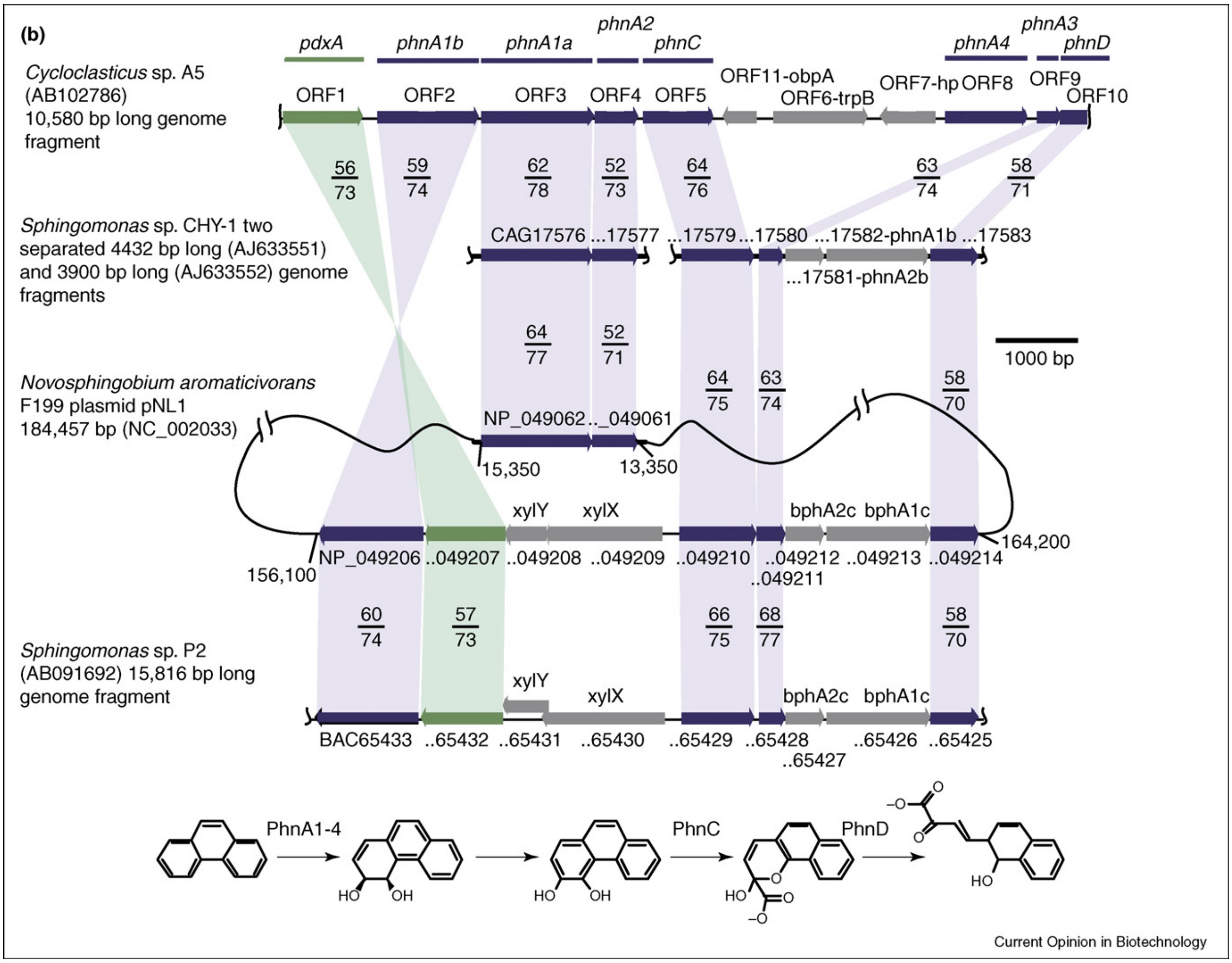
A similarly ubiquitous genetic organization also exists for determinants specifying degradation of aromatic hydrocarbons. Analysis of the organization of gene clusters in *Cycloclasticus* spp. for the degradation of (poly)aromatic hydrocarbons revealed a cluster of six open reading frames *xylXMKGC1C2* that specifies utilization of all three forms of xylene [51]. The cluster also exhibits similar organization to the catabolic determinants of the PAH-degrading freshwater β-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

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: *alk*B1, alkane monooxygenase; *alk*G, rubredoxin; *alk*J, alcohol dehydrogenase; *alk*H, aldehyde dehydrogenase. **(b)** Organization of gene clusters of PAH degradation pathways of *Cycloclasticus* sp. A5 and PAH-degrading α-proteobacteria. *pdxA* genes predicted to code for the pyridoxal phosphate biosynthesis enzyme are coloured in green, PAH degradation genes are in blue. Percentages of protein identity/similarity of polypeptides from *Cycloclasticus* sp. A5 with those of the PAH-degrading α-proteobacteria are shown. Gene designations: *phn*A1, iron–sulfur protein (ISP) α subunit of PAH dioxygenase; *phn*A2, ISP β subunit of PAH dioxygenase; *phn*A3, Rieske-type [2Fe–2S] ferredoxin, *phn*A4, NADH-ferredoxin oxidoreductase; *phn*C, extradiol [3,4-dihydroxyphenanthrene] dioxygenase; *phn*D, 2-hydroxy-2*H*-benzo[*h*]chromene-2-carboxylate isomerase.

Figure 3. (Continued).



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reductase, respectively, encode the initial enzyme of the pathway, PAH dioxygenase [52]. As can be seen in Figure 3b, the genetic organization of the *phn* gene cluster of the γ-proteobacterial *Cycloclasticus* is similar to that found in several α-proteobacteria. The *phn* gene cassette of *Novosphingobium aromaticivorans* F199 is located on a large plasmid, pNL1 [53]. Interestingly, the *pdx*A gene, which specifies an enzyme involved in the biosynthesis of the coenzyme pyridoxyl phosphate (PLP), is clustered and co-oriented with *phn*A1b, seemingly a characteristic feature of *phn*-like cassettes (Figure 3b). The broad PAH substrate range characteristic of *Cycloclasticus* might 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, has been detected in *C. oligotrophus* [51]. This indicates that *Cycloclasticus* spp. might degrade not only diverse (poly)aromatic hydrocarbons, but also chlorinated derivatives thereof — consistent with their ubiquity in marine environments.

Marine OHCB in oil-degrading communities

Several studies have shown that an influx of oil in a marine site causes population densities of OHCB 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 remaining compounds that are more difficult to degrade [54,55•] (see also [2•,17,20] for reviews). 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 [56] studied community population shifts in microcosms of superficial sediments from a chronically polluted area of the Milazzo Harbour oil refinery, Sicily. The samples 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 a 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 OHCB, and *Alcanivorax* spp. in particular, were obtained in a mesocosm study involving a 14000 L bioreactor [57]. 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. *Alcanivorax* spp. was observed when samples were spiked with the branched aliphatic hydrocarbon pristine, and *Cycloclasticus* spp., when spiked with PAHs [33]). A complementary study to analyse 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 [58]. *Oleispira* was also shown to be present in oil-degrading microbial communities in other microcosm studies performed at low temperatures. Brakstad and Bonaunet [59] identified *Oleispira* spp. in such a study, in addition to several species of γ-*Proteobacteria* (e.g. *Psychromonas* spp.) and *Bacteroidetes*. Similarly, Gerdes *et al.* [38] emphasized the importance of *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 [60].

Although revealing, such microcosm studies as closed systems are reductionist and artificial, as they lack the complexity, 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 Penninsula), from the Mediterranean Sea, and from the North Sea, are identical in terms of their small subunit rRNA gene sequences, but exhibit distinct substrate preferences and temperature requirements (MM Yakimov, PN Golyshin, unpublished). 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. Moreover, 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 the mitigation of oil spills has been discussed elsewhere [2•,18]. It has since become apparent, however, that such bacteria might 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 PHAs [61•], even though the normal food storage compounds found in this strain and other OHCB (*Marinobacter* and *Thalassolituus)* are triacylglycerols and wax esters [62]. 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 the need for the costly extraction of PHA from intracellular granules using environmentally problematic solvents. Thus, the mutant provides a potential *Alcanivorax* cell factory for biopolymer production [62,63].

As OHCB are relatively recent discoveries, and have a novel physiology, they 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 the retrieval of novel enzymes [64•]. Functional screening of the library resulted in the identification of five groups of carboxylesterases. The most abundant group, 'Oil2'-type polypeptides (GenBank AJ811965), were affiliated with *Marinobacter* α-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) comprised the 'O.23' carboxylhydrolase, which possessed the carboxylhydrolase B domain and was distantly related (~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 several important drug precursors). The most unusual protein, 'O.16', demonstrated the highest enantioselectivity (*E* 126[S]; 98% enantiomeric excess) ever reported for the ester of the important chiral synthon solketal. This enzyme, from an as yet unknown hydrocarbon degrader, contains three catalytic serine residues in two domains exhibiting distinct activities — a thioesterase and a carboxylesterase. The enzyme also has a unique adaptive structure:function characteristic that involves radical changes in its tertiary and/or quarternary structure in response to changes in environmental physical-chemical conditions.

Conclusions

A significant input to our knowledge on the genomics of HCB is expected to result from the genomic analysis of another hydrocarbon-degrading marine γ-*Proteobacterium*, *Marinobacter aquaolei* VT-8, also known in the literature 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=genomeprjandcmd=searchandterm=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 the ~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 γ-*Proteobacterium* belonging to the true OHCBs for which the 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. Crucial to such studies are functional genomic analyses of OHCBs to yield *inter alia* understanding of OHCB responses to the varying stresses experienced in the marine environment (e.g. osmotic, solute, temperature, pressure, UV irradiation, etc.), which is of crucial importance for the development of effective strategies for the biomitigation of oil spills. Genetic tools that have been developed to study other γ-proteobacteria, although not as developed as those for *E. coli*, work well for the study and manipulation of OHCB and enable the 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. The behaviour of individual functional members of such networks will also need to be assessed, as nicely exemplified by a recent case study on the anaerobic biodegradation of organic matter by a complex microbial community [65•]. 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 (e.g. viral, prokaryotic and 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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- •• of outstanding interest

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