Genomics of deep-sea and sub-seafloor microbes

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Over two-thirds of the surface of the earth is covered by oceans, which have an average depth of about 3800 m. As each drop of ocean water contains $> 10^5$ cells, the $> 10^{30}$ microbial cells in the ocean represent the largest reservoir of microbes on earth (Whitman et al., 1998). Communities of bacteria, archaea, protists and unicellular fungi account for most of the oceanic biomass and metabolism. Marine microbes are known to play an essential role in the global cycling of nitrogen, carbon, oxygen, phosphorous, iron, sulfur and trace elements (Karl, 2007).

The largest metagenome sequencing projects undertaken to date involved surface seawater samples from the Sargasso Sea (Venter et al., 2004) and the Global Ocean Sampling (GOS) (Yooseph et al., 2007) expeditions conducted by Craig Venter and colleagues. The GOS analysis has shown that the numbers of newly discovered protein families in microbes have not yet reached a plateau, and in fact the curve is still rising. This means that there are many more functionalities to be discovered. This surface seawater metagenome sequencing did not take into account the piezophiles (= pressure-loving) and so it would be reasonable to assume that this is still a large and generally untapped source of potentially useful enzymes and products.

The microbes of the very deep differ from those isolated from shallow waters in several aspects. The GOS analysis suggests that near-surface microorganisms do not need chemolaxis, flagellae or pili to actively swim around in search of food, as they rely mostly on the plentiful O$_2$, CO$_2$ and sunlight for photosynthesis. Yet this is not the case for the deep-sea piezophilic psychrophiles. Deep-sea environments are characterized by low temperature (1–2°C), high pressure (1 MPa for every 100 m), high-salt and low-nutrient conditions. Pressure affects various aspects of metabolism in very different ways (Simonato et al., 2006). Little light penetrates below 500 m, the presence of food is scarce and many organisms have adapted to survive long periods without nutrition. Prokaryotic adaptation to deep sea habitats has been reviewed by Simonato and colleagues (2006) and Lauro and Bartlett (2008). It is assumed that life here will be heterotrophic and largely supported by influxes of nutrients coming down from the more ‘fertile’ waters above. Nutrients slowly reach the seabed in the form of dead whales, crustaceans, fish, kelp, wood and their debris. These all carry the microbiota that was associated with them as they sank to the ocean bed (Egan et al., 2008). These surface-associated microbes can to a certain extent also adapt to the changing conditions in which they now find themselves.

Microbial isolates or communities of some deep-sea ‘oases’ such as hydrothermal vents in the seafloor (Nakagawa and Takai, 2008) or whale falls (Tringe et al., 2005) have been sampled and studied, but these are exceptions which we do not address here.

Databases and computing tools

Marine microbe researchers are highly organized with respect to storing and sharing their data. The Community Cyberinfrastructure for Advanced Marine Microbial Ecology Research and Analysis (CAMERA) aims to develop global methods for monitoring microbial communities in the ocean and their response to environmental changes. The CAMERA’s database (http://camera.calit2.net) includes environmental metagenomic and genomic sequence data, associated environmental parameters (‘metadata’), pre-computed search results, and software tools to support powerful cross-analysis of environmental samples (Seshadri et al., 2007) (Fig. 1). The CAMERA includes the Sargasso Sea and GOS expedition data, as well as a vertical profile of marine microbial communities collected at the Hawaii Ocean Time-Series station ALOHA by Ed DeLong and his research team at MIT. In addition, the MetaLook software has been developed for visualisation, analysis and comparison of marine ecological genomic and metagenomic data with respect to habitat parameters (http://www.megx.net/metalook) (Fig. 2).

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Fig. 1. Example of a BLAST search by CAMERA.
(Meta)genome sequencing of deep-sea microbes

Thanks to initiatives by the Gordon and Betty Moore Foundation, nearly 200 genomes of culturable marine bacteria have been sequenced since 2004 (http://www.moore.org/microgenome/). Table 1 summarizes deep-sea and sediment (meta)genome sequencing projects.

Most of the sequenced culturable microorganisms from the deep-sea are Alteromonadales from the Gammaproteobacteria. Unique properties of sequenced deep-sea microbes are that they all have a high ratio of rRNA operon copies per genome size, and that their intergenic regions are larger than average (Lauro and Bartlett, 2008). These properties are characteristic of bacteria with an opportunistic lifestyle and a high degree of gene regulation to respond rapidly to environmental changes when searching for food. A large number of genes are found for synthesis of mono- and polyunsaturated fatty acids and membrane unsaturation in these deep dwelling microorganisms, as they need to maintain membrane fluidity at low temperature and high pressure (Simonato et al., 2006). They also contain a larger than average repertoire of transport proteins for scavenging different types of food. Large numbers of proteins are encoded for chemotaxis, flagellar assembly and motor function to allow them to hunt for dissolved and particulate organic matter, also called ‘marine snow’ (Azam and Long, 2001; Kiorboe and Jackson, 2001). The latter consists mainly of diffuse gels with a variety of organisms living together in biofilms and communities highly dependent upon each other for survival. The marine snow can sink thousands of metres into the sea. This brings down to the seabed a lot of organic material, nitrogen and phosphorus, helping to sustain the communities present at depth (for a review see Azam and Malfatti, 2007). In order to make use of this food source the

Fig. 2. The starting point of MetaLook: the world map showing genomics and metagenomics sampling sites. Clicking on a location will provide all genomics and meta data.
| Phylum          | Order                  | Organism                | Isolation                                           | Depth (m)   | Reference/data               |
|-----------------|------------------------|-------------------------|-----------------------------------------------------|-------------|-----------------------------|
| Euryarchaeota   | Methanococci           | Methanococcus aeolicus  | Nankai-3 Deep marine sediment, Nankai Trough Japan | NC_009635  |                             |
| Euryarchaeota   | Methanomicrobia        | Methanotheon marisnigri | JR1 Sediment, Black Sea                             | NC_009051  |                             |
| Chlorobia       | Chlorobia              | Chlorobium phaeobacteroides BS1 | Chemoicline, Black Sea                             | NC_010831  |                             |
| Proteobacteria  | Gammaproteobacteria    | Shewanella piezotolerans | WP3 Sediment, west Pacific                         | 1914        | Wang et al. (2008)          |
| Proteobacteria  | Gammaproteobacteria    | Alteromonas macleodii   | DSM 17117 Seawater, Urania Basin, Mediterranean     | 3500        | NZ_ABCQ00000000             |
| Proteobacteria  | Gammaproteobacteria    | Moritella sp. PE36      | Patton escarpment, off San Diego, Pacific          |             |                             |
| Proteobacteria  | Gammaproteobacteria    | Shewanella benthica KT99, PT99 | Tonga-Kermadec Trench, Pacific                   | 5800        | NZ_AABC00000000             |
| Proteobacteria  | Gammaproteobacteria    | Shewanella woodyi MS32  | Sediment, Strait of Gibraltar, Mediterranean       | 5110        | NC_010506                   |
| Proteobacteria  | Gammaproteobacteria    | Shewanella lohica PV-4  | Iron-rich mat, Naha Vents, Hawaii                  | 1325        | NC_009092                   |
| Proteobacteria  | Gammaproteobacteria    | Shewanella sp. W3-18-1  | Sediment, Washington, Pacific                      | 997         | NC_008750                   |
| Proteobacteria  | Gammaproteobacteria    | Shewanella violacea DSS12 | Sediment, Ryukyu Trench, Philippine Sea         | 5110        | nakasone@hiro.kindai.ac.jp   |
| Proteobacteria  | Gammaproteobacteria    | Photobacterium profundum SS9 | Sulu Trough                                | 2500        | Vezzi et al. (2005)         |
| Proteobacteria  | Alphaproteobacteria    | Roseobacter sp. SK221-2-6 | Arabian Sea                                      | 2500        | NZ_AAYC00000000             |
| Proteobacteria  | Epsilonproteobacteria  | Sulfitobacterium autotrophica OK10 | Deep-sea sediment, Mid-Okinawa Trough, Japan   | 1100        | microbes@cubas.jgi-psf.org  |
| Proteobacteria  | Zetaproteobacteria     | Mariflustrum ferrooxydans PV-1 | Loihi Seamount, Hawaii                           | 1050        | Takami et al. (2002)        |
| Firmicutes      | Bacilli                | Oceanobacillus iheyensis HTE831 | Deep sea mud, Iheyu ridge, Okinawa Japan       | 1050        |                             |
| Firmicutes      | Bacilli                | Carnobacterium sp. AT7  | Aleutian Trench                                  | 2500        | NZ_ABHH00000000             |
| Firmicutes      | Clostridia             | Carboxydribachium pacificum JM | Okinawa Trough                                 | 1500        | NZ_ABXP00000000             |
| Metagenomes     | Unclassified           | Marine anammox community | Deep sea                                          | 520         | sgtringe@lbl.gov            |
| Metagenomes     | Unclassified           | Marine archael anaerobic oxidation of methane communities | Sediment, Eel River Basin, Mendocino California |             | Hallam et al. (2004)        |
| Metagenomes     | Unclassified           | Marine planktonic communities | North Pacific Ocean, ALOHA station, Hawaii     | 500–4000    | DeLong et al. (2006)        |
| Metagenomes     | Unclassified           | Marine microbial communities | Seep water masses of the North Atlantic     | 500–4121    | Sogin et al. (2006)         |
| Metagenomes     | Unclassified           | Marine planktonic communities | Mediterranean Sea, Ionian Km3 Station     | 3010         | Martin-Quadrado et al. (2007) |
| Metagenomes     | Unclassified           | Sediment microbial communities | Sub-seafloor, Peru Margin                      | 1229        | Biddle et al. (2008)        |

Adapted from the GOLD database (http://www.genomesonline.org; December 2008).
microorganisms have fine-tuned hydrolytic enzymes and nutrient uptake systems. Very few metagenomics studies of deep-sea communities have been reported so far, and include only deep water samples from the North Pacific Gyre ALOHA station (DeLong et al., 2006) and the Ionian Sea in the Mediterranean (Martin-Cuadrado et al., 2007), and (sub)seafloor samples from the Peru Margin (Biddle et al., 2008) and the Eel Basin off the coast of California (Hallam et al., 2004) (Table 1). The cold (2–5°C) North Pacific deep waters were found to contain Deferribacteres, Planctomycetaceae, Acidobacteriales, Gemmatimonadaceae, Nitrospira, Alteromonadaceae, and SAR11, SAR202 and Agg47 bacterial clades (DeLong et al., 2006); these microbial communities were enriched in genes encoding transposases, pilus synthesis, protein transport, polysaccharide and antibiotic synthesis, the glyoxylate cycle, and urea metabolism, relative to surface communities. The warmer (14°C) deep Mediterranean waters contained mainly Proteobacteria, but also Actinobacteria, Firmicutes, Planctomycetales, Chloroflexi, Bacteroidetes, Acidobacteria, and also Crenarchaeota (Martin-Cuadrado et al., 2007). Enriched functional categories were again for pilus, polysaccharide and antibiotic synthesis, as well as peptide and amino acid transporters, while genes involved in degradation of complex biopolymers and xenobiotics were also abundant. The high percentage of genes encoding dehydrogenases, and among them cox genes, suggested that aerobic CO oxidation may play a role in deep seas as additional energy source. Genomes update (Gomes and Steiner, 2004).

**Discovery of novel enzymes from deep-sea microbes**

Marine enzyme biotechnology can offer novel biocatalysts with properties like high salt tolerance, hyperthermostability, barophilicity, cold adaptivity, and ease in large-scale cultivation (reviewed by Debashish et al., 2005). Metagenomics strategies are powerful tools to identify enzymes with novel biocatalytic properties from unculturable members of microbial communities (Ferrer et al., 2009; Steele et al., 2009). The GOS project discovered hundreds of truly novel protein and enzyme families from marine surface microbes for which no function is yet known (Yooseph et al., 2007). Deep-sea and sediment microbes should provide an enormous reservoir of low-temperature and high-pressure adapted enzymes. Both sequence-based and function-based screening approaches have been used to identify enzymes with potentially interesting biocatalytic activities from cultured deep-sea microbes as well as uncultured metagenomes (Kennedy et al., 2008; Kobayashi et al., 2008) (Table 2).

For instance, highly salt-tolerant and pressure-tolerant esterases were identified in a metagenome expression library generated from microbes isolated from a deep-sea hypersaline anoxic basin in the Eastern Mediterranean (Ferrer et al., 2005). The amino acid content of proteins in psychrophilic piezophiles is also different from that in mesophiles. There are more polar amino acids in the proteins, resulting in a loss of rigidity and increased structural flexibility for enhanced catalytic activity. The adaptive properties of psychrophilic enzymes are high specific activity, relatively low temperature optima and high thermostability (Gomes and Steiner, 2004).

Table 2. Enzymes from deep-sea microbes.

| Enzyme(s)          | Producing organism(s) | Isolation                         | Reference                  |
|--------------------|-----------------------|-----------------------------------|----------------------------|
| α-Amylase          | Nocardiosis sp.       | Deep sea sediment                 | Zhang and Zang (2008)      |
| Alkane hydroxylases| Metagenome            | Deep sea sediment                 | Xu et al. (2008)           |
| β-Lactamases       | Metagenome            | Cold-seep sediment of seamount    | Song et al. (2005)         |
| Cellulase          | Pseudoaltermonas sp.  | Deep-sea sediment                 | Zeng et al. (2006)         |
| Esterases          | Metagenome            | Deep-sea hypersaline anoxic basin | Ferrer et al. (2005)       |
| Esterase (alkaline)| Metagenome            | Deep-sea sediment                 | Park et al. (2007)         |
| Lipase             | Metagenome            | Deep-sea sediment                 | Hardeman and Sjoling (2007) |
| Lipase             | Metagenome            | Deep-sea sediment                 | Jeon et al. (2009)         |
| Quinol oxidase     | Shewanella sp. strain | Deep-sea sediment                 | Qureshi et al. (1998)      |
| Protease (alkaline)| Pseudomonas strain    | Deep-sea sediment                 | Zeng et al. (2003)         |
| Proteases (neutral, alkaline) | Pseudoaltermonas sp. | Deep-sea sediment                 | Xiong et al. (2007)        |
| Protease (alkaline)| Pseudoaltermonas sp.  | Deep-sea sediment                 | Chen et al. (2007)         |
| Various            | Metagenome            | Deep sub-seafloor sediment core   | Kobayashi et al. (2008)    |

Adapted from Kennedy and colleagues (2008).
A major challenge will be to develop alternative hosts and their associated vectors for heterologous expression of genes from the diverse phyla existing in the deep-sea ecosystem (Ferrer et al., 2009).

Applications

Cold-adapted enzymes are advantageous for waste decomposition in cold environments, for food processing, flavour enhancement (He et al., 2004) and preservation, and for processes that require the rapid inactivation of enzymatic reactions (Huston et al., 2000; O’Brien et al., 2004). Piezophilic enzymes could be useful for food sterilization at high pressure and low temperature, improving preservation of flavour and colour. Halophilic enzymes can be applied for non-aqueous reactions, as they have better thermostability and other unique properties in organic solvents, and could be used in anti-fouling coating and paint industries (Yebra et al., 2004), but also for synthesis of optically active substances. Finally, many deep-sea bacteria can synthesize interesting chemical compounds, such as omega-3 polyunsaturated fatty acids that are considered useful in reducing the risk of cardiovascular disease, and polyketides (Siezen and Khayatt, 2008) which could be used as novel antibiotics.

Acknowledgements

This project was carried out within the research programme of the Kluyver Centre for Genomics of Industrial Fermentation which is part of the Netherlands Genomics Initiative/ Netherlands Organization for Scientific Research.

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