Chapter 4
The Deep Subseafloor and Biosignatures

Frédéric Gaboyer, Gaëtan Burgaud, and Virginia Edgcomb

Abstract A critical issue in astrobiology is "where to look for present or past life?" and which types of environments could be relevant, i.e. environments associated with high probabilities to (have) support(ed) life and preserve(d) biosignatures. Due both to the large reservoir it represents and to its protective effect against harmful surface conditions, for example radiation, oxidation, the subsurface is of considerable interest in astrobiology. On Earth, living microorganisms have been documented buried in the subsurface up to depths of several kilometers, demonstrating that the deep subsurface can be inhabited by complex microbial communities for millions of years and offering astrobiologists the possibility to better understand how life could be supported, and what kind of biosignatures could be expected, in the subsurface of other planetary bodies. In this chapter we present general trends in the microbial ecology of deep subsurface environments and their peculiar conditions, with a focus on sedimentary microbial ecosystems. We provide a case study of the Canterbury Basin subseafloor as an analogue, subsurface ecosystem on extraterrestrial planetary bodies, and discuss analytical methods for studying microbial lifestyles and preservation in that ecosystem.

F. Gaboyer (✉)
Centre de Biophysique Moléculaire, CNRS, Orléans, France
e-mail: frederic.gaboyer@cnrs-orleans.fr

G. Burgaud
Université de Brest, Laboratoire Universitaire de Biodiversité et Ecologie Microbienne (EA3882), ESIAB, Plouzané, France
e-mail: gaetan.burgaud@univ-brest.fr

V. Edgcomb
Woods Hole Oceanographic Institution, Woods Hole, MA, USA
e-mail: vedgcomb@whoi.edu

B. Cavalazzi, F. Westall (eds.), *Biosignatures for Astrobiology*, Advances in Astrobiology and Biogeophysics, https://doi.org/10.1007/978-3-319-96175-0_4

4.1 The Deep Biosphere: An Unseen World of Contrasting Habitats

4.1.1 Definition

The deep biosphere (DB) is represented by a wide range of terrestrial and aquatic environments (Fig. 4.1), including (1) the terrestrial DB: deep aquifers, mines and caves; (2) the aquatic DB: the deep ocean, its (sub)surface sediments and igneous crust, and lacustrine habitats (e.g., subglacial lakes).

The DB encompasses a huge variety of ecological niches characterised by site-specific physical and geochemical conditions that are difficult to unify in a single definition. Additionally, deep environments share characteristics, such as a relative disconnection from the surface, the presence of chemosynthetic-based food webs, and atypical environmental conditions. Since the depth of subsurface habitats varies between sites, it seems unreasonable to set a depth threshold defining the DB.

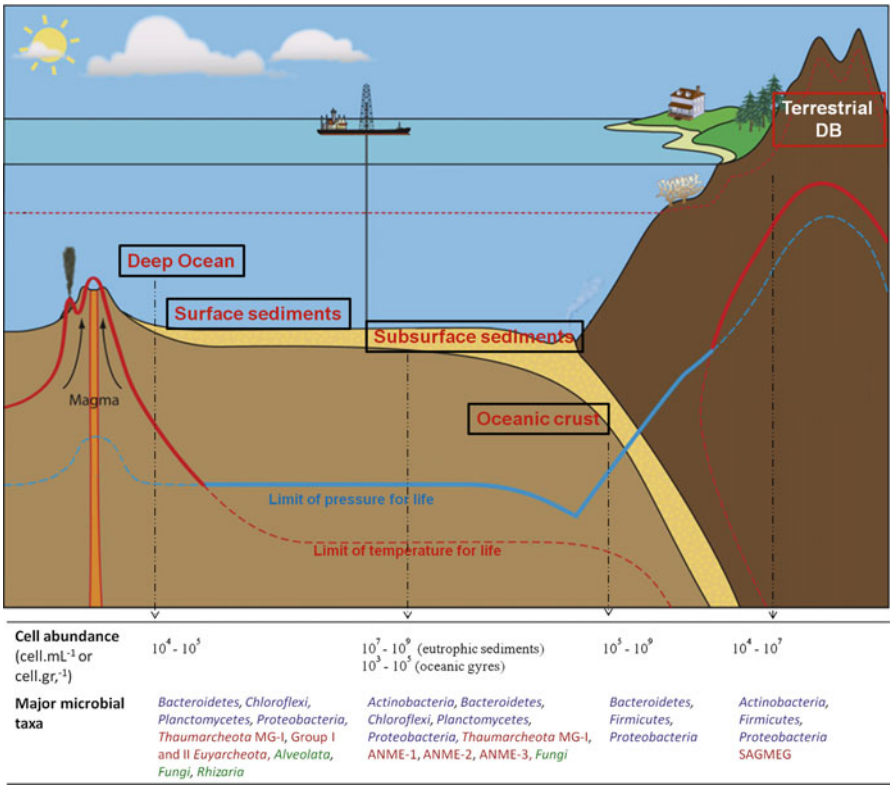

Fig. 4.1 Schematic representation of the DB and associated cell abundances and taxa per type of habitat. Source: Adapted from Oger and Jebbar (2010)

Considering the subseafloor, Teske and Sørensen (2008) proposed "*sediment layers with distinct microbial communities that lack a microbial imprint of water column communities should be considered deep subsurface*." Extending this idea to other environments, we suggest as a guideline for defining the DB that it represents *environments that are physically isolated from the surface with microbial communities that are distinct from those in shallow layers of the Earth and with food webs that can function independently from solar energy*. However, as mentioned above, the disconnection of the DB from the surface is relative, as highlighted by several lines of evidence, including that microbial cell numbers decrease in subsurface sediments in areas of lower sedimentation, or that archaeal $\delta^{13}C$ isotopic signatures can overlap with cells of photosynthetic origin (Biddle et al. 2006). The similarity between fungal DNA sequences from the deep subseafloor and DNA sequences of terrestrial Fungi (Rédou et al. 2015) also indicates that the surface biosphere contributes to shaping the subsurface biosphere.

4.1.2 Environmental Conditions of Subsurface Environments

The marine deep subsurface represents a vast reservoir for life on Earth, extending several hundred to more than a thousand meters into deeply buried sedimentary habitats and even further down into igneous crust hydrated by fluid flows. The environmental conditions of the DB are hostile to most forms of life, favouring the selection of so-called extremophiles. We note that physicochemical extremes interacting in a synergetic way (Harrison et al. 2013) should not be considered individually when characterising the DB.

4.1.2.1 Temperature and Pressure

An increasing temperature gradient with depth creates a progressive succession of thermal conditions for psychro/meso/thermophiles. This gradient is estimated to average 20 °C/km in the oceanic crust, 25 °C/km in the terrestrial subsurface and 30–60 °C/km in subsurface sediments (Turcotte and Schubert 2002). Near-surface sediments typically have a temperature range of 2–4 °C (the temperature of the deep ocean). The upper temperature limit for life is 122 °C under high pressure for *Methanopyrus kandleriir* (Takai et al. 2008) and 113 °C at atmospheric pressure for *Pyrolobus fumarii* (Blöchl et al. 1997), implying that life in the DB could persist up to 2–5 kilometers below the seafloor (kbsf). However, it should be kept in mind that the laboratory experiments in which these organisms grew were conducted under energy-replete conditions. Thus, considering the low amount of energy available for cells in the DB, microorganisms are unlikely to survive in the DB to their experimentally-determined upper temperature limits.

Large differences in temperature and pressure exist due to the heterogeneity of local environments, e.g. hydrothermal fluids emanating from vents near tectonic and

volcanic boundaries can reach 450 °C and circulate through subseafloor fractures. Geothermal activity in terrestrial systems also provides local hot circulating groundwater, rendering some horizons ideal for (hyper-)thermophiles. Thermophiles have been obtained from Japanese gold mine samples at 350 m below land surface and at 80 °C (Inagaki et al. 2003), whereas the terrestrial subsurface at similar depth in crust not particularly influenced by hydrothermal circulation tends to be considerably cooler.

Pressure has various effects on microbial physiology (for a thorough review, see Oger and Jebbar 2010) but is unfortunately not routinely measured during sampling. The pressure gradient occurring with depth depends on the material properties of rocks (e.g., porosity, compaction degree). In oceanic systems, in addition to the pressure gradient of sediments (15–25 MPa/km) and oceanic crust (30 MPa/km), the water column imposes a pressure gradient of 10 MPa/km (Dziewonski and Anderson 1981). Since seafloor is located at an average depth of 3800 m, the basal pressure of marine surface sediments is ~38 MPa. Extreme piezophilic or piezotolerant organisms can survive and remain active in buried habitats of the DB. *Colwellia* BNL-1 and *Methanocaldococcus jannaschii* grow optimally at 93 MPa/10 °C (Deming et al. 1988) and 75 MPa/86 °C (Jones et al. 1983), respectively. The maximum pressure limit compatible with cell activity reported to date is 120 MPa for *Pyrococcus yayanossi* (Birrien et al. 2011), which corresponds to a depth limit of 3.3–5.5 kbsf in sediments (25–15 MPa/km) and of 2.7 kbsf (30 MPa/km) in the oceanic crust, considering a basal pressure of 38 MPa at the seafloor.

4.1.2.2 Activity of Water and Porosity

The low water activity (A_w) within the DB leads to physiological challenges for cells, such as osmotic pressure changes and difficulties in membrane transport. Enzymatic activities are also inhibited due to the catalytic roles played by H_2O. To cope with this, cells must respond with energy-consuming processes in environments with minimal energy sources (Jørgensen and Boetius 2007).

Evolution has also produced life forms adapted to extremely dry environments, thus life may remain active in the DB down to A_w ~0.64, based on reported microbial growth in laboratory experiments with A_w, as low as 0.635 and 0.64 for haloarcheal and fungal species, respectively (Stevenson et al. 2015). Note that, even though materials can be humid, the lack of liquid water available for biological activity can result in very low A_w. In deeper subsurface habitats, originally humid sediments give way to progressively drier and compacted rocks. Clay-rich sediments for instance are typically replaced with sedimentary rocks, such as marlstone and limestone.

Porosity is also a major parameter controlling life in the DB and is correlated to subseafloor cell abundance (Parkes et al. 2000). Indeed, pores are microniches in which fluid circulation produces chemical gradients, thus, potentially supplying

microorganisms with nutrients, electron donors and acceptors and enabling microbial growth. The low porosity and the absence of macro-fractures limit the presence of water in the DB. Although porosity and water activity stresses are related, we note that in some places there is simply insufficient space for buried cells. Black shales are an excellent illustration of this point since, despite high organic content, they are barely inhabited by cells due to their compaction.

4.1.2.3 Energy Sources

It is estimated that less than 1% of organic matter (OM) in the oceans is accumulated in the subseafloor and is largely consumed in the first centimeters of sediments (Orcutt et al. 2011). Since most subseafloor sediments are located in the low-productivity abyssal regions of the open ocean, the OM content of sediments in those regions can be extremely low. The maturation of OM with depth during diagenesis produces refractory carbon unavailable to most microorganisms (Burdige 2007). The marine DB is thus an extreme oligotrophic environment, and very low metabolic rates and long generation times are expected for the cells inhabiting it (Jørgensen and Boetius 2007; Hoehler and Jørgensen 2013; Jørgensen and Marshall 2016).

As mentioned above, circulating water can provide chemical gradients that supply microbial metabolisms with electron donors (mainly organic matter, hydrogen, reduced sulphur compounds, reduced iron compounds, and ammonium) and acceptors (mainly oxygen, nitrate/nitrite, manganese and iron oxides and sulphite/sulphates). Since redox couples with the highest free energy are preferentially consumed in the top layers of sediments, this leads to zonation where oxygen, nitrate, manganese, iron and sulphate are consumed in a successive and sequential pattern (DeLong 2004). As a consequence, only traces of electron donors and acceptors are present in deeper horizons in the absence of fluid circulation.

Other natural processes can supply microbial metabolisms with alternative energy sources: serpentinisation produces methane and hydrogen during the interaction of water with olivine and pyroxenes, whereas radiolysis of water leads to hydrogen production from ^{236}U, ^{232}Th and ^{40}K radioactivity, a process that was shown to strongly support microbial life in subsurface sediments of the South Pacific Gyre (D'Hondt et al. 2009).

Specific lifestyles may be particularly adapted to the nutrient-poor conditions of the DB. For example, it has been reported that a single Firmicute species, *Candidatus Desulforudis audaxviator*, inhabits a gold mine at 2.8 kms depth with a lifestyle well-suited to long-term isolation from the photosphere, based on inorganic carbon and nitrogen fixation, sulphate reduction, or complete anabolism (Chivian et al. 2008). This kind of independent lifestyle is an elegant example of how life could autonomously persist in the subsurface of extraterrestrial, basaltic, planetary bodies, such as Mars.

4.2 Tools to Detect Subsurface Biosignatures

4.2.1 Contamination Issues

As discussed in Lever et al. (2006), there are significant challenges in eliminating contamination of core samples collected from below the seafloor. The huge amount of surface seawater injected into the borehole is a major potential source of contamination. Various protocols can be employed at sea to clean the exteriors of collected rock, and/or to remove exterior material from rock or sediment cores.

DNA sequencing approaches are required to evaluate the extent to which core material may still be contaminated, for instance sequencing different negative controls (kit and the drilling fluids). Signatures of organisms found to be identical in both the drilling fluid and rock/sediment samples may be a sign of contamination, and the most prudent approach is to eliminate those signatures from downstream analyses.

Chemical tracers and microsphere beads are also injected into the seawater drilling fluids to assist microbiologists in the evaluation of potential contamination but inconsistencies in the distribution of microbeads have limited their utility for this purpose. Perfluoromethylcyclohexane (PFMC) is the most commonly used perfluorocarbon tracer by microbiologists (Smith et al. 2000). The sample is flamed upon recovery to volatilize the PFMC, to release it into the air and thereupon quantify it using gas chromatography. Any PFMC detected on the interior of a sample means that drilling fluid could have penetrated into the sample, and thus it is likely contaminated. A problem with this approach is that PFMC is extremely volatile and, once a few samples have been run, it can generally be detected in most samples because of airborne contamination. A less volatile tracer, perfluoromethyldecalin (PFMD) was recently tested during the Expedition 360 (South West of the Indian ridge), of the International Ocean Discovery Program (IODP). PFMD was found to produce more reliable results (Edgcomb and Sylvan unpublished data). Another approach used a modified microsphere tracer dispersed in an aqueous fluorescent pigment (Friese et al. 2017).

4.2.2 Sensitive Analytical Methods

The most challenging aspect of investigating life in the subsurface is that, despite its apparent ubiquity in the DB, cell numbers can be extremely low (Kallmeyer et al. 2012; Parkes et al. 2014), particularly in crustal samples. This necessitates extremely sensitive analytical procedures for the detection of cells and cell activities (Lomstein et al. 2012).

Stable- and radio-isotope tracer-based techniques represent one possible approach based on sample incubation under conditions mimicking in situ conditions. This has been used to measure microbial activities such as sulphate reduction

(Jørgensen and Boetius 2007), methanogenesis, methane oxidation (Orcutt et al. 2010), and hydrogenase activity (Nunoura et al. 2009). The resulting data can be influenced by artefacts associated with physicochemical changes that occur during, and following, sample recovery. In this respect, the development of new in situ mass and laser absorption spectrometers hold great promise (Cowen et al. 2012).

To quantify active subsurface microbial populations using microscopy, dead cells must be differentiated from living cells and their biosignatures. Some of the first studies of DB sediments applied epifluorescence microscopy to quantify cell abundances but were restricted to organic-rich sediments with high cell abundance. Recent advances that allow cells to be separated from sediments (Kallmeyer et al. 2008) with counting automation (Morono et al. 2009) have resulted in a downward revision of estimated total cells in the DB sediments (Kallmeyer et al. 2012).

Most assessments of microbial diversity within the marine DB have utilized PCR amplification of target genes, most commonly small subunit ribosomal RNA (SSU rRNA) genes, from extracted DNA. Because these extracts include DNA from active, inactive but viable, and dead cells, and also extracellular DNA, they do not exclusively represent active cells. This has spurred researchers to pursue analyses targeting the more labile RNA molecule, with its reverse transcription into complementary DNA (cDNA), that can be then analyzed either as a metatranscriptome, capturing functional information from active communities (Orsi et al. 2013b; Pachiadaki et al. 2016), or as a template to amplify target genes (Orsi et al. 2013a; Rédou et al. 2014). Curiously, nucleic acids extracted from subsurface marine sediments exhibit signatures of taxa that are clearly not endogenous to this habitat, indicating that preservation of DNA and small subunit ribosomal RNA preservation of bacterial spores, fossilized diatoms with silica-rich frustules, cysts of protists and pollen or spores from plants up to 2.7 My ago is possible in sediments and rocks with reduced water content (Orsi et al. 2013a). The degree to which DNA is preserved in these cells is unknown, however the presence of this paleome challenges the interpretation of deep subsurface molecular data making the design of integrated approaches highly desirable.

Lipid biomarkers also provide good targets for the detection of deeply buried cells with high sensitivity. High-pressure liquid chromatography coupled to mass spectrometry has been applied to lipids extracted from subsurface hydrothermal samples (Sturt et al. 2004), and can also be used to identify particular microbial groups. Intact polar lipids are thought to derive from viable intact cells, as polar head groups are hydrolyzed after cell death in sedimentary environments within days, although their persistence as head groups for millions years may also be possible in immature sediments (Schouten et al. 2013).

Polar head groups devoid of core lipids reflect fossil remains and can, thus, provide information about past microbial communities. Ultra-sensitive triple quadrupole mass spectrometry may also be required for the analysis of low biomass samples, such as crustal rocks. Paired with confocal Raman spectroscopy analyses, a non-destructive method, it may be possible to visualize microfossils or living cells in such samples since confocal Raman spectroscopy allows the recognition of living and fossil microorganisms in rock material (Foucher et al. 2010, 2015).

4.3 Microbiology of Subsurface Sediments Using the Canterbury Basin (CB) as a Case Study

4.3.1 Drilling Expeditions and Environmental Context

The IODP Leg 317 (December 2009) expedition took place in the CB, offshore New Zealand. The *JOIDES Resolution* drilled four holes (U1352A to D) at a water depth of 344 m at site U1352, ~75 km from shore. A total core of 1927 m was recovered, spanning the Holocene to late Eocene (the deepest layers aged 32 Ma) and divided into three units of clay and calcareous sandy mud (unit I, from surface to 709 mbsf), sandy marlstone and limestone (unit II, 709 to 1852 mbsf) and limestone and cherts (unit III, 1852 to 1927 mbsf). Abundance in calcareous nannofossils and planktonic foraminifera provides good biostratigraphic age controls (Fulthorpe et al. 2011).

Investigation of environmental conditions throughout the core showed that temperatures at the bottom were ~60 °C. Measured pH varied from 7 to 8 and fluctuated with sulphate reduction, methanogenesis and possibly carbonate precipitation. Sediment porosity ranged from 40% near the surface to >10% in the deepest layers, and TOC varied from 0.25% to 0.75%. Gas contents ranged between 1 to 10 ppmv (ethane and propane) and to 10^4 to 10^2 ppmv (methane) throughout the core (Fulthorpe et al. 2011).

4.3.2 Cell Abundance

Although initial estimates showed that microorganisms of the DB represented 35% to 47% of Earth's total biomass (Whitman et al. 1998), these data were largely obtained in organic-rich sediments close to continents and associated with high sedimentation rates. Considering the sedimentation rates and distances from continents, Kallmeyer et al. (2012) offered more reserved and realistic estimates of the subseafloor biomass of 3.10^{29} cells and 4.10^{15}g C in total, representing 0.6% of Earth's total biomass. Taking into account recent studies that have reported intact cells down to 1922 mbsf (Ciobanu et al. 2014), this estimate can be further revised at $5.39.10^{29}$ cells (Parkes et al. 2014). Without doubt, estimates of cell abundances are likely to be amended in coming years, especially now that microbial life has been documented down to 2.5 kbsf with atypical cell concentrations along the core (Inagaki et al. 2015).

Although the presence of spores and of viral-like particles (VLPs) in the DB is poorly studied, it is of great importance since it is known that spores can persist under stressful conditions and can reactivate after several million years (Vreeland et al. 2000). Lomstein et al. (2012) quantified spores (dipicolinic acid) and bacterial (muramic acid and D-amino acids) biomarkers, finding that "*endospores are as abundant as vegetative cells and microbial activity is extremely low, leading to*

microbial biomass turnover times of hundreds to thousands of years", highlighting the critical, but underestimated, role of spores in microbial ecology of the DB.

Viral-like particles (VLPs) strongly impact upon the carbon cycle and microbial populations. Bacteriophages were first revealed by induction of the viral cycle on strains isolated from subsurface sediments (Engelhardt et al. 2011), showing head-tailed morphologies, typical of *Myoviridae* and *Siphoviridae*. The presence of VLPs in subsurface sediments has been confirmed by in situ studies down to 320 mbsf, where VLP cell abundances in sediments are 10^9 cells cm^{-3} (Engelhardt et al. 2014) and virus/cell ratios from 1 to 10 suggest ongoing viral production (Engelhardt et al. 2012).

4.3.2.1 The Canterbury Basin Subseafloor Case Study

In the CB, mean cell numbers decrease with depth from about $1.5 \times 10^6 \pm 4.7 \times 10^4$ cells cm^{-3}at the surface to $2.5 \times 10^4 \pm 4.9 \times 10^3$ cells cm^{-3} within the deepest samples (1922 m.b.s.f.) (Fig. 4.2a). The detection limit was estimated to be 2.94×10^3 cells cm^{-3}. The detected cells were very small (0.3–0.8 μm). This depth profile is consistent with the general depth distribution of prokaryotic cells from other subsurface sediments, showing a logarithmic decrease in cell number with depth. No spores or viruses were observed, despite the fact that genes for sporulation and prophages were detected using metagenomic analyses (Gaboyer et al. 2015).

4.3.3 Evolution of Diversity with Depth

Complex microbial communities have been revealed in subsurface sedimentary habitats, including representatives from all three domains of life (Bacteria, Archaea and mostly Fungi among the micro-Eukarya). As the taxonomic composition appears to reflect oceanic regions and site-specific conditions (Parkes et al. 2014), it is difficult to highlight any diversity pattern. Nevertheless, for Bacteria the dominant phyla are Chloroflexi, Proteobacteria, Planctomyces, the candidate phylum Atribacteria, Firmicutes and Actinobacteria; for Archaea, the dominant members are Crenarchaeota with the Lokiarchaeota (previously MBG-B), Euryarchaeota with the Hadesarchaea (previously SAGMEG) and Marine Benthic Group-D (MBG-D, an archaeal group within the Thermoplasmatales) and Thaumarchaeota with the Bathyarchaoeta (previously MCG) and the Marine Group I (MG-1). These acronyms reflect that, to date, most subsurface microbial groups have largely escaped cultivation attempts, and their phylogeny remains uncertain at the phylum-level (Brochier-Armanet et al. 2008).

There is certainly a pattern in the diversity of subsurface microbial communities, controlled by organic matter supply and redox status (Durbin and Teske 2011). Whereas the patterns of distribution in Bacteria tend to be relatively similar between

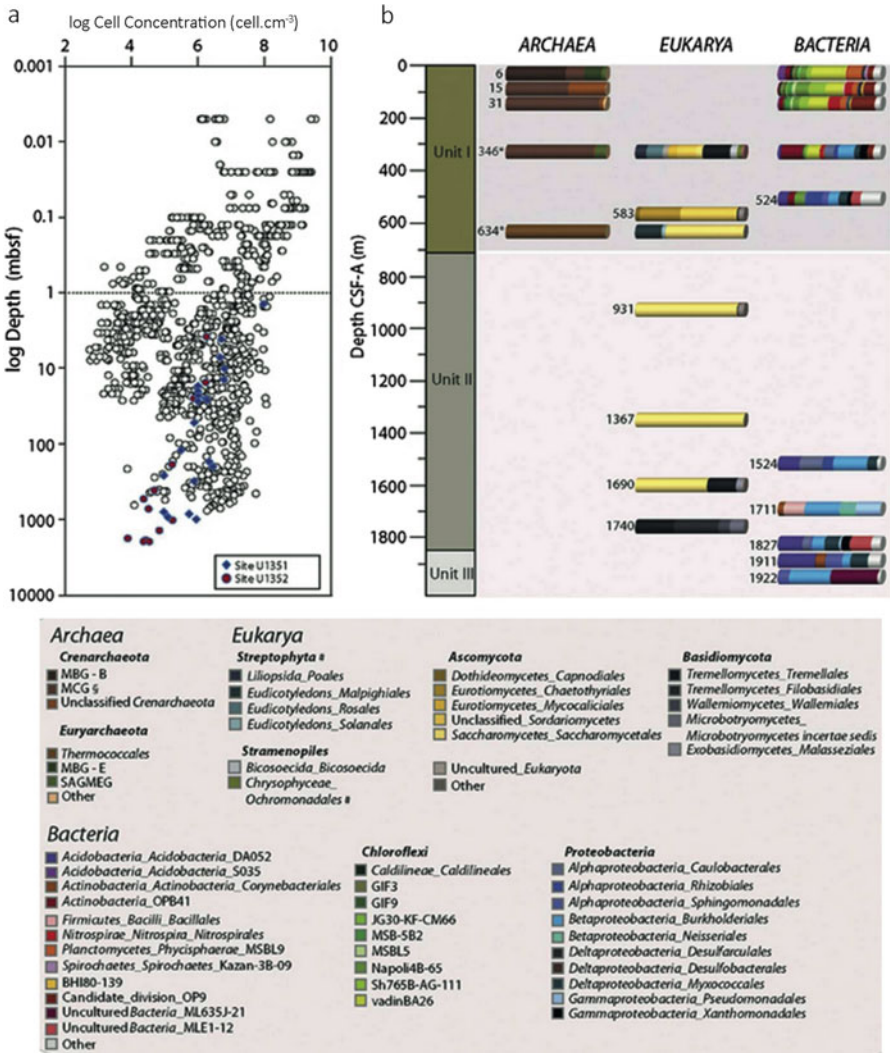

Fig. 4.2 (**a**) Cell counts at two sites in the Canterbury Basin (red and blue circles) compared to general cell counts (white circles) in sub-seafloor sediments. Source: Adapted from Kallmeyer et al. (2012). (**b**) Phylum Class/Order distribution of archaeal, eukaryotic and bacterial 16S/18S rRNA gene-tag sequences (based on SILVA111 classification) from OTUs containing >100 sequences. Source: Adapted from Ciobanu et al. (2014)

sites, contrasting results on archaeal diversity strongly suggest that each subsurface site, characterised by its own environmental factors, has its own archaeal fingerprint.

Nevertheless, without standardized methodologies (extraction methods, targeted genes, primers, sequencing technologies and bioinformatics pipelines), it is difficult to draw any firm conclusions about the presence of particular prokaryotic groups and their relative abundance in different subsurface habitats.

While the focus of debate has mainly centered on bacterial or archaeal dominance, less is known about the eukaryotes. Culture-independent approaches targeting microeukaryotes suggest their presence and activity in deep subsurface sediments on the basis of DNA and SSU rRNA (Edgcomb et al. 2011; Orsi et al. 2013a; Ciobanu et al. 2014; Rédou et al. 2014). Fungal sequences appear to dominate the fraction of microeukaryote signatures, with some fungal representatives having been successfully isolated from Northwestern Pacific margin samples up to 2457 mbsf (Liu et al. 2016).

Irrespective of the domain of life concerned, patterns in microbial diversity with depth are certainly not random but governed by the selection of cells that can survive starvation, such that a progressive selection of adapted survivors occurs from the surface to the depth, as proposed recently on the basis of DNA sequencing and genome analysis (Starnawski et al. 2017). Both the abundance of cells and their diversity within microbial communities decreased down the CB core (Fig. 4.2b). Multidimensional statistical analyses highlighted that, among environmental factors, changes in microbial taxa were mainly defined by depth (Ciobanu et al. 2014). The number of OTUs (Operational Taxonomic Units) per domain was very low with 198, 16 and 40 unique bacterial, archaeal and eukaryotic OTUs being detected, most of them retrieved in the first hundred meters of the CB core. Both iTAG and qPCR data indicated that recovered archaeal SSU rRNA genes decreased between 6 and ~600 mbsf. Archaeal sequences could not be amplified and sequenced below 634 mbsf. Sequences belonging to Archaea were mainly affiliated to Lokiarchaeota (MBG-B) and Bathyarchaeota (MCG). Bacterial SSU rRNA genes were detected in samples from the full length of the CB core. Sequences obtained from each depth layer decreased in diversity with depth, with ~100 OTUs recovered in samples analyzed from between 6 and 31 mbsf, ~40 OTUs in samples from 346 and 524 mbsf, and fewer than 20 OTUs in samples from 634 to 1922 mbsf (Ciobanu et al. 2014). OTUs were mainly affiliated to *Chloroflexi* and *Proteobacteria* and exhibited a shift from *Chloroflexi* dominance above 600 mbsf to Proteobacterial dominance below 343 mbsf. Signatures of *Planctomycetes*, *Nitrospirae* and *Atribacteria* dominated the first layers, whereas *Acidobacteria* and *Firmicutes* were retrieved below 600 mbsf.

Other snapshots of DB diversity revealed close distributions in the patterns of particular taxa. For example, in deep coal-bed sediments up to 2457 mbsf (Inagaki et al. 2015) *Chloroflexi* sequences were also observed in quantity at shallow depths. *Chloroflexi* dominated bacterial communities above 600 mbsf for IODP Leg 317 (CB) samples and above 364 mbsf for IODP Leg 337 (Northwestern Pacific margin). In the deeper layers of the CB, a shift occured to *Actinobacteria*, *Proteobacteria* and *Firmicutes* as the dominant groups (Ciobanu et al. 2014; Inagaki et al. 2015).

Concerning Eukarya, few 18S sequences affiliated to bacterivorous protists were retrieved. Consistent with previous studies, Fungi appeared to be the most frequently detected micro-eukaryotes in the CB, particularly *Ascomycota* and *Basidiomycota*.

Eukaryotic richness dropped off gradually along the core, indicating a strong vertical structuring of the community.

A complementary approach specifically targeting fungal DNA confirmed low fungal diversity in CB sediments with poor overlap between fungal OTUs at the different depths suggesting a spatial differentiation of fungal communities (Rédou et al. 2014). Curiously, most fungal OTUs were phylogenetically related to ubiquitous terrestrial Fungi. Using the same samples, a culture-based approach generated 183 fungal isolates (Rédou et al. 2015). Consistent with molecular data, numerous fungal isolates were related to well-known Fungi in terrestrial environments, raising ecological questions regarding the ability of buried terrestrial Fungi to adapt to deep subsurface conditions. Indeed, this similarity points to the contribution of the surface biosphere to the subsurface biosphere and that terrestrial fungi "trail" into the sub-seafloor, undergoing a selection of the most adapted cells to burial and starvation (Starnawski et al. 2017).

We note that describing bacterial, archaeal or eukaryotic groups as abundant at specific depths does not mean they are dominant in the sediment core. During the dynamic burial process, species that are more resistant to starvation will survive (Starnawski et al. 2017) and, thus, communities will change their size and diversity (alpha diversity). The continuous selection of cells with burial makes the notion of microbial dominance relative and, therefore, extending the dominance of one group to other depths incongruent.

4.4 Activity *Versus* Viability

Given the biogeochemical heterogeneity of subsurface sediment horizons, crustal rocks and fluid flows, it stands to reason that there is great heterogeneity also in activity levels of subsurface microbiota, even at different depths along the same core length. From more active cells close to the surface to deeply buried cells, a community shift occurs with the selection of cells more adapted to subsurface starvation (Starnawski et al. 2017). Whether or not they are adapted to oligotrophy, subsurface cells can be more or less active in sediments. This question of activity *versus* dormancy remains a critical issue for understanding the microbial ecology of the subseafloor.

DNA and RNA preservation is possible in deep subsurface habitats, and hence signatures of active cells can be confused with signatures of inactive or even dead cells. To tease apart the signal of live cells from this pool of molecules, an integrated approach is required that blends information from "meta-omics" and culturing with physiological screening of microbial isolates and microscopy to visualize cells.

Cell activity in the deep subsurface biosphere has been detected using different approaches, including microscopy (dividing cells) (Pachiadaki et al. 2016), direct measurement of intermediates in cell processes (D'Hondt et al. 2002), analysis of intact polar lipids (Lipp et al. 2008) and DNA or RNA (Orsi et al. 2013a, b; Rédou et al. 2014; Pachiadaki et al. 2016). The flux of electron donors and acceptors, deriving notably from photosynthesis in overlying seawater or terrestrial

environments, mainly controls the level of cell activity of subseafloor microorganisms. Organic carbon content in sub-seafloor provinces varies from 0.09 to >12 wt.% (Schrenk et al. 2010), is higher in continental margin and lower in the open ocean, leading to, respectively, higher and lower cell activity.

Successful cultivation of subsurface isolates (e.g., Batzke et al. 2007; Biddle et al. 2005) does not provide information about the in situ activity of cells but only on their viability once they have been brought to labs. Microbial groups in extremely low abundance in the subseafloor can be the dominant groups among isolates if they have simpler cultivation requirements and more easily reproduced. The description of cells on "physiological standby" maintaining the capacity to metabolize (Morono et al. 2011), supports the idea of a gap between in situ and cultivation-based diversities.

Metatranscriptomics helps to differentiate the metabolic signatures coming from living cells from those of living but inactive or dead cells. Transcripts involved in prokaryotic cell division were found in the Peru Margin subseafloor (Orsi et al. 2013b). Gene transcripts associated with motility may also be a signal of active cells, since motility in the subsurface may be limited by extremely low available energy (Jørgensen and Marshall 2016). In subsurface sediments from the Peru Margin, flagellar protein signatures were detected but, logically, appeared to decrease with depth in most, but not all, samples analysed (Orsi et al. 2013b). Similarly, transcripts of genes associated with cell-cell interactions, particularly those involved in competition for precious resources or pili formation, also indicated that not all cells are dormant in the subsurface. Pili are hair-like appendages on the surfaces of cells that involved in bacterial adherence, conjugation or movement (see Piepenbrink and Sundberg 2016 for a review) and are therefore another sign of interactions between cells and their environment.

4.4.1 The Canterbury Basin Subseafloor Case Study

Successful cultivation of prokaryotes and Fungi inhabiting CB sediments showed that at least a fraction of in situ microbes were viable.

Moreover, an integrated approach designed for studies of the subseafloor of the CB strongly supported the idea that part of the microbial community was not only present but also active. For example, a poly-A targeted metatranscriptome from a 345 mbsf sample revealed fungal transcripts involved in growth, cell division, sporulation, and catalytic activities within different classes of enzymes such as hydrolases, suggesting that Fungi play important roles in biogeochemical cycles of the DB. Bacterial and archaeal transcripts also showed that these buried cells are active in situ.

Finally, microscopic observations of reproductive fungal structures are direct evidence for in situ cell activity and growth (Pachiadaki et al. 2016).

4.5 Possible Metabolisms

Since microbiota exist in the majority of subseafloor locations investigated to date, they must have adaptations for survival in these energy-limited habitats. This is demonstrated by culture-based studies that show the ability of subsurface microbes to survive on extremely low energy fluxes (Jørgensen and Marshall 2016).

The activities of subsurface microorganisms were studied extensively in samples from the Peru Margin (PM) during ODP Leg 201. Since redox couples with highest free energy are first consumed, chemical profiles exhibit predictable zonation at the PM, with depletion of the highest energy yielding species in shallow depths (Fig. 4.3). Indeed, dissolved electron acceptors such as sulphate (SO_4^{2-}) and nitrate (NO_3^-) exhibit subsurface depletion, whereas metabolic products such as dissolved inorganic carbon (CO_2 + HCO_3^- + CO_3^{2-}), ammonia (NH_3 + NH_4^+), sulphide (H_2S + HS^-), methane (CH_4), manganese, and iron consistently exhibited concentration maxima deep in the drilled sediments (D'Hondt et al. 2004). As a consequence, processes thought to be active at the PM mainly include organic carbon oxidation, ammonification, methanogenesis, methanotrophy, sulphate reduction, manganese reduction and, to a lesser extent, iron reduction, production and consumption of formate, acetate, lactate, hydrogen, ethane, and propane. Dissimilatory

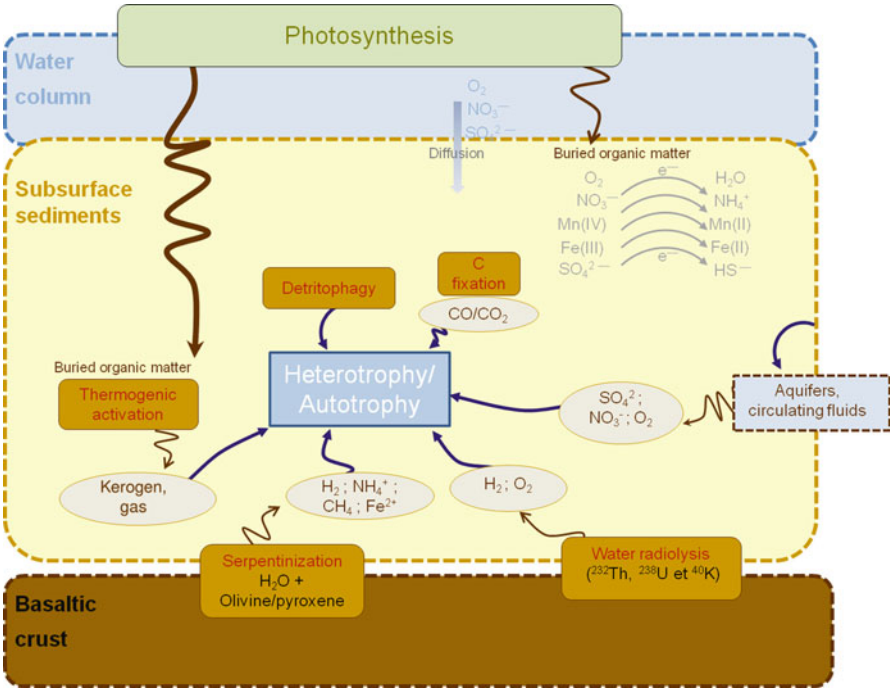

Fig. 4.3 Schematic representation of microbial metabolisms occurring in the subseafloor. Source: F Gaboyer

sulphate reduction may represent the dominant form of energy production in subseafloor sediments, as suggested by pore-water sulphate concentrations (D'Hondt et al. 2004) and by the occurrence of dissimilatory reductase transcripts that reflect biogenic sulphate reduction.

Concerning heterotrophy, with the exception of particularly organic-rich zones, microbial populations must survive on recalcitrant (low reactivity) and diminished concentrations of organic material that has escaped remineralisation by other cells during seafloor deposition and subsequent burial (Burdige 2007). The most labile molecules are rapidly removed after burial. Buried gas hydrates, organic rich layers, and sulphate/methane interfaces may provide new pools of labile organic molecules for cells. At depth, CH_4, hydrocarbons, acetate, H_2 and CO_2 may be released from buried organic material at thermogenic temperatures, providing additional sources of carbon and energy (Parkes et al. 2014). Serpentinisation, the aqueous alteration of ultramafic rocks, produces high-energy microbial substrates, such as organic compounds, H_2 and CH_4, and thus contributes to the maintenance of life in the DB (Fig. 4.3) (Schulte et al. 2006).

Metatranscriptomic analyses used to examine the microbial activities in samples from 6 to 95 mbsf at the PM (Pachiadaki et al. 2016) showed a majority of transcripts associated with enzymes involved in carbohydrate, amino acid and lipid metabolism. Most of these enzymes can participate both in the catabolism and anabolism of organic materials. The successful detection of transporters in all samples, considered as a proxy for active catabolism, indicated heterotrophy in the PM subseafloor. This strengthens previous suggestions that amino acids may be important sources for carbon and nitrogen metabolisms in the subsurface (Lloyd et al. 2013). The recovery of a wide range of transcripts for various carbohydrate transporters also suggests that carbohydrates serve as additional energy sources for microbes in the shallow samples at PM (Pachiadaki et al. 2016).

4.5.1 The Canterbury Basin Subseafloor Case Study

Metabolisms within the CB subsurface, as detected by metagenomics and metatranscriptomics, were mostly represented by heterotrophy, notably through fermentation and respiration of nitrite or sulphate, and by possible autotrophy based on CO fixation. Indeed, cultivation of Bacteria and Archaea from the CB highlighted capabilities for fermentation, while attempts to cultivate true methanogens and sulphate reducers were unsuccessful (Ciobanu et al. 2014). Cultivating subsurface microorganisms is very challenging and, hence, molecular approaches provide valuable information about metabolisms occurring in CB sediments.

Heterotrophy in the CB was also supported by metatranscriptomic and metagenomic data that revealed transcripts and genes for various sugar, amino acid and lipid transporters, as well as transcripts and genes for their degradation (Gaboyer et al. 2015; Pachiadaki et al. 2016). A possibly significant archaeal

contribution to amino acid fermentation was indicated by metatransciptomic analyses. Metagenomic analyses showed the importance of extracellular substrate uptake by revealing the presence of more than 100 secreted extracellular peptidase genes. The expression of many genes involved in enzyme production, putative exoenzymes, indicates that these enzymes may be involved in organic carbon turnover in the DB and, more precisely, in degradation of refractory organic matter. This is consistent with recent microbial biomass quantification data on CB samples (Zhu et al. 2016), indicating that a significant proportion of microbial debris is preserved (e.g., prokaryotic necromass) in the CB and, thus, available for decomposers, including Fungi. Among other carbon sources, the presence of haloacid dehalogenase (*had*) genes suggests the potential for utilization of organohalide compounds.

Autotrophic pathways in the CB subseafloor have only been suggested by the detection of genes encoding the reductive acetyl-CoA pathway, based on monoxide carbon fixation. Determining the phylogeny of metabolic genes recovered within genomic fragments showed that Chloroflexi, Euryarcheota and Crenarcheota were all capable of fermentation, that Crenarcheota may use halogens as a carbon source, and that Euryarcheota may be capable of autotrophic CO fixation (Gaboyer et al. 2015).

Considering anoxygenic respiration, the description of *dsrA* (sulphate reduction) and *rdh* (reductive dehalogenase) genes as well, as transcripts for nitrite reductase (denitrification), indicated that sulphate, nitrite and organohalide compounds may, respectively, serve as electron acceptors within the CB subsurface (Pachiadaki et al. 2016).

4.6 Physiological Potential

The term “sociomicrobiology” reflects the idea that the Earth hosts subtle microbial lifestyles, leading to group behaviour and deep physiological changes in processes, such as quorum sensing, biofilm formation or motility. The possibility exists that such complex microbial lifestyles also occur in the DB. The few studies performed to date on subsurface samples combining metagenomics, metatranscriptomics and culture-based approaches are starting to provide answers to this question.

In the PM subseafloor, transcripts of genes associated with reproduction, cell-cell interactions, particularly those involved in competition for precious resources, for adhesin or pili all indicate complex cell-cell interactions. In all samples analysed from the PM, gene transcripts for toxin/antitoxin production, and with antibiotic production and resistance, were detected (Pachiadaki et al. 2016), such as transcripts for beta-lactamases used by bacteria to defend their peptidoglycan-synthesizing machinery against the toxic effects of penicillin derivatives (Fig. 4.4). Given the signaling and communicating roles of antibiotics in natural environments, this strengthens the idea of interacting cells in the subsurface.

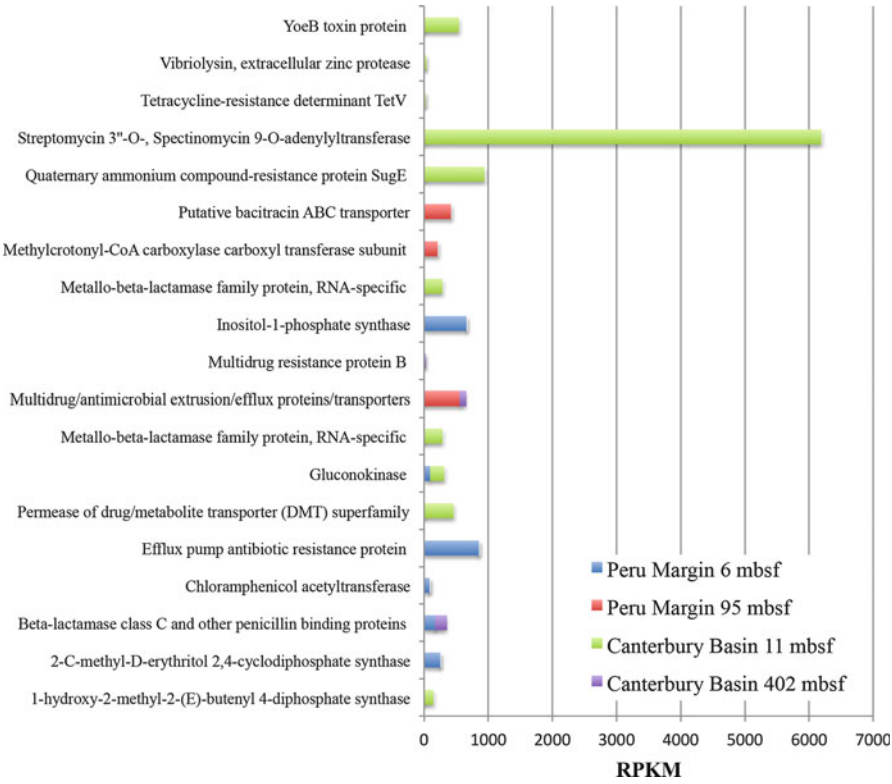

Fig. 4.4 Relative expression (presented as RPKM values) of genes associated with toxin and antimicrobial/antibiotic synthesis/resistance in the Canterbury Basin and Peru Margin subseafloors. Source: Reprinted with permission from Pachiadaki et al. (2016)

Beyond energy limitation and the subsequent selection of most adapted cells, there are many stressors for life in the deep subsurface, including pressure, temperatures, heavy metal toxicity, etc. Little is known about how microorganisms adapt to these co-occurring extremes, when the availabilities of electron acceptors/donors enables cells to deploy stress responses.

A study of two cultured hydrothermal vent Archaea reveals that growth depends on a delicate balance between stressors. As long as pH was mildly acidic or neutral and not more acidic than pH 5.5, *Thermococcus* and *Pyrococcus* could tolerate a wider range of pressures (up to 850 atm) and temperatures (up to 100 °C) than those likely to be found in the subsurface (Edgcomb et al. 2007). Pressure and elevated temperature changes are known to simultaneously induce a wide range of both heat shock and cold shock proteins, possibly as an attempt to repair the effects of changing pressure on membrane integrity/fluidity or macromolecule stability (Oger and Jebbar 2010). The extent to which these proteins play a role in the survival of DB communities is difficult to assess based on metatranscriptomics because

depressurization and other physicochemical changes that occur in samples during recovery from the subsurface likely bias the expression of genes for these proteins.

Early studies of hydrothermal vents revealed the potential for high concentrations of heavy metals in vent fluids, hinting that the subsurface biosphere must also be able to cope with occasionally high heavy metal concentrations that are known to interfere with cell function by binding to vital macromolecules, and to control microbial community composition (Ravikumar et al. 2007). A study of three hydrothermal vent archaeal cultures revealed that the addition of sulphide improved the high toxicity of free metal cations of Zn, Co, and Cu by the formation of dissolved metal-sulphide complexes and precipitates (Lloyd et al. 2005). This suggests that the presence of sulphides in subsurface habitats may help microorganisms to cope with heavy metals concentrations. Metatranscriptome analyses of subsurface samples support the notion that heavy metals can stress subsurface microbial populations. The expression of general ion transport-related genes was detected in PM subsurface samples including genes affiliated with siderophore biosynthesis, magnesium and iron transporters, and chelatases that may be associated with detoxification activities (Pachiadaki et al. 2016). We also note that some microbes in anoxic sediments can respire through the coupled reduction of iron or sulphur and toxic metals, such as arsenic (Reyes et al. 2008).

4.6.1 The Canterbury Basin Subseafloor Case Study

Similar trends for complex cell-cell interactions and resistance to heavy metals and drugs were also highlighted in the CB subseafloor. For example, pilus assembly and flagellar motility are suggested by the occurrence of genes/mRNAs for pilus formation and flagellar assembly up to 345 mbsf, indicating that energy limitations do not completely prevent such processes from occurring in the CB. This is an important point considering that pore spaces offer support for cell adhesion (Gaboyer et al. 2015; Pachiadaki et al. 2016).

The ability of buried microorganisms to adapt their activity to changing environmental conditions is a crucial selective advantage. In the CB, numerous genes involved in environmental sensing and gene expression regulation were detected, including transcriptional regulators, sigma/anti-sigma factors, adenylate and diguanylate cyclases involved in the synthesis of signaling molecules, two-component systems and the GTP pyrophosphokinase involved in adaptation to starvation (stringent response) (Gaboyer et al. 2015). However, these DNA-data cannot be used as evidence for the in situ expression of adaptive genes but only for an in situ potential for such adaptations. Stress response strategies were more strongly suggested by mRNA-based studies (Pachiadaki et al. 2016). These included responses to pressure and low A_w (osmolytes accumulation and synthesis, regulation of membrane composition), and high temperatures (chaperonins, heat shock

proteins) or oxidative stress (glutathione enzymes, thioredoxins). Pachiadaki et al. (2016) reported the apparent importance of drug/metal resistance genes (drug/metals membrane transporters, cobalt-zinc-cadmium proteins). Genes for sporulation were also detected in CB samples, suggesting that some microbes in the CB subseafloor can sporulate and wait for more favourable conditions. High-throughput physiological screening of fungal isolates in CB samples down to 1884 mbsf revealed a physiological shift from terrestrially-adapted to marine-adapted styles along the core for some specific species. Such an integrated approach clearly demonstrates that some Fungi may be able to adapt to subsurface conditions, while some may be dormant as spores (microbial zombies) and others may not survive. This corroborates the idea of a progressive selection of microbial groups adapted to long term starvation during the burial of surface communities in deeper sediments (Starnawski et al. 2017).

Polyketide synthases, nonribosomal peptide synthetases and terpene synthases are known as enzymes producing secondary metabolites, some with bioactive properties, involved in complex cell interactions. Genes coding for these enzymes were found in 167 of the 176 fungal isolates obtained from CB samples up to 765 mbsf, as well as their mRNAs up to 345 mbsf (Rédou et al. 2015; Fig. 4.5).

Detection of fungal genes/mRNAs for antibiotic production and prokaryotic genes/mRNAs for resistance suggest complex cell interactions, notably between Fungi and bacteria in the subsurface of the CB. This situation is not unique to the CB since similar microbial lifestyles were described in the subsurface of the well-studied PM (Orsi et al. 2013b). To confirm this hypothesis, fungal isolates from the CB were screened for their ability to synthesize antibacterial compounds (Navarri et al. 2016). A trend was observed whereby the proportion of fungal isolates producing antimicrobial compounds decreased with increasing depth and significantly correlated with bacterial diversity richness. From an ecological perspective, this suggests that, since shallow sediment depth layers are associated to higher microbial diversity, complex interactions between microorganisms are more pronounced in these regions than in deeper zones. This paves the way for more integrated studies implementing metabolomics to better understand interactions between microbial communities in the DB. The above-described results show that the deep subseafloor sediments are not free of sociomicrobiology, as genes and mRNAs for processes of microbial competition, adhesion or communication were detected. However, such interactions are only possible if there is sufficient energy and available nutrients. Indeed the main environmental constraints and limiting factor for life in the subseafloor remains long-term starvation. Here again, we note that the surface community composition progressively changes during burial, with community composition along the sediment core controlled by the selection of microorganisms adapted to oligotrophy, as experimentally confirmed (Starnawski et al. 2017). The lack of gene expression studies in the subseafloor still prevents the comparison of physiologies between depth and sites and scientists still have to wait for more investigations to be done in the future to clearly answer that point.

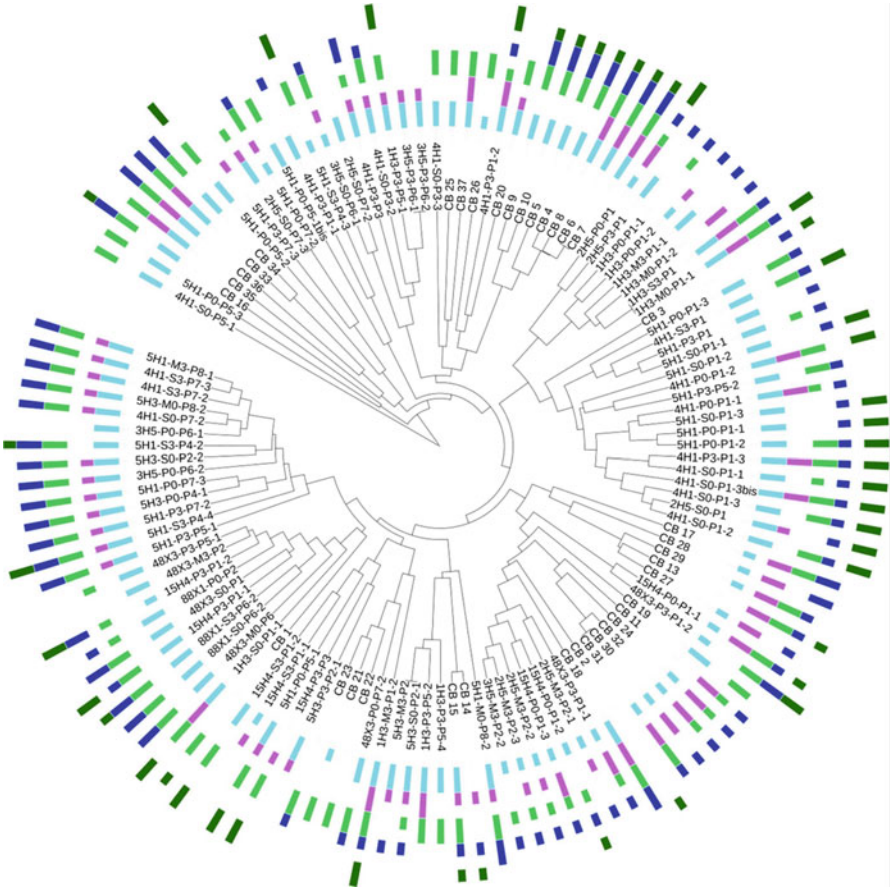

Fig. 4.5 Presence/absence of genes encoding type I and III Polyketide Synthase (PKSI, PKSIII), non ribosomal peptide synthetase (NRPSs), PKS-NRPS hybrids, and terpene synthase (TPS) in filamentous fungi of the CB subseafloor. PKSI, light blue; PKSIII, pink; NRPS, light green, PKS-NRPS hybrid, dark blue; TPS dark green. Occurrences are presented using an aligned multivalue bar chart (short bar, only one gene; long bar, several genes). Source: Adapted from Rédou et al. (2015)

References

Batzke A, Engelen B, Sass H et al (2007) Phylogenetic and physiological diversity of cultured deep-biosphere Bacteria from Equatorial Pacific Ocean and Peru Margin sediments. Geomicrobiol J 24:261–273

Biddle J, House CH, Brenchley JE (2005) Microbial stratification in deeply buried marine sediment reflects changes in sulfate/methane profiles. Geobiology 3(4):287–295

Biddle J, Lipp J, Lever M, Lloyd K, Sørensen K, Anderson R, Fredricks H, Elvert M, Kelly T, Schrag P (2006) Heterotrophic Archaea dominate sedimentary subsurface ecosystems off Peru. Proc Natl Acad Sci USA 103(10):3846–3851

Birrien J-L, Zeng X, Jebbar M et al (2011) *Pyrococcus yayanosii* sp. nov., an obligate piezophilic hyperthermophilic archaeon isolated from a deep-sea hydrothermal vent. Int J Syst Evol Microbiol 61:2827–2881

Blöchl E, Rachel R, Burggraf S et al (1997) *Pyrolobus fumarii*, gen. and sp. nov., represents a novel group of archaea, extending the upper *temperature* limit for life to 113 C. Extremophiles 1:14–21

Brochier-Armanet C, Boussau B, Gribaldo S, Forterre P (2008) Mesophilic Crenarchaeota: proposal for a third archaeal phylum, the Thaumarchaeota. Nature Reviews Microbiology 6 (3):245–252

Burdige DJ (2007) Preservation of organic matter in marine sediments: controls, mechanisms, and an imbalance in sediment organic carbon budgets? Chem Rev 107:467–485

Chivian D, Brodie EL, Alm EJ et al (2008) Environmental genomics reveals a single-species ecosystem deep within earth. Science 322:275–278

Ciobanu M-C, Burgaud G, Dufresne A et al (2014) Microorganisms persist at record depths in the subseafloor of the Canterbury Basin. ISME J 8:1370–1380

Cowen JP, Copson DA, Jolly J et al (2012) Advanced instrument system for real-time and time-series microbial geochemical sampling of the deep (basaltic) crustal biosphere. Deep-Sea Res I Oceanogr Res Pap 61:43–56

D'Hondt S, Rutherford S, Spivack A (2002) Metabolic activity of subsurface life in deep-sea sediments. Science 295:2067–2070

D'Hondt S et al (2004) Distributions of microbial activities in deep subseafloor sediments. Science 306:2216–2221

D'Hondt S, Spivack AJ, Pockalny R et al (2009) Subseafloor sedimentary life in the South Pacific Gyre. Proc Natl Acad Sci USA 106:11651–11656

DeLong E (2004) Microbial life breathes deep. Science 306:2198–2200

Deming J, Somers L, Straube W et al (1988) Isolation of an obligated barophilic bacterium and description of a new genus *Colwellia* Gen-nov. Syst Appl Microbiol 10:152–160

Durbin AM, Teske A (2011) Microbial diversity and stratification of South Pacific abyssal marine sediments. Environ Microbiol 13:3219–3234

Dziewonski AM, Anderson DL (1981) Preliminary reference Earth model. Phys Earth Planet Inter 25:297–356

Edgcomb VP, Molyneaux SJ, Böer S et al (2007) Survival and growth of two heterotrophic hydrothermal vent archaea, *Pyrococcus* strain GB-D and *Thermococcus fumicolans*, under low pH and high sulfide concentrations in combination with high temperature and pressure regimes. Extremophiles 11:329–342

Edgcomb VP, Beaudoin D, Gast R et al (2011) Marine subsurface eukaryotes: the fungal majority. Environ Microbiol 13:172–183

Engelhardt T, Sahlberg M, Cypionka H et al (2011) Induction of prophages from deep-subseafloor bacteria: phages in the deep-subseafloor. Environ Microbiol Rep 3:459–465

Engelhardt T, Sahlberg M, Cypionka H et al (2012) Biogeography of *Rhizobium radiobacter* and distribution of associated temperate phages in deep subseafloor sediments. ISME J 8:1503–1509

Engelhardt T, Kallmeyer J, Cypionka H et al (2014) High virus-to-cell ratios indicate ongoing production of viruses in deep subsurface sediments. ISME J 8(7):1503–1509

Foucher F, Westall F, Brandstätter F et al (2010) Testing the survival of microfossils in artificial martian sedimentary meteorites during entry into Earth's atmosphere: the STONE 6 experiment. Icarus 207:616–630

Foucher F, Ammar M-R, Westall F (2015) Revealing the biotic origin of silicified Precambrian carbonaceous microstructures using Raman spectroscopic mapping, a potential method for the detection of microfossils on Mars. J Raman Spectrosc 46:873–879

Friese A, Kallmeyer J, Kitte JA, et al the ICDP Lake Chalco Drilling Science Team and the ICDP Towuti Drilling Science Team (2017) A simple and inexpensive technique for assessing contamination during drilling operations: a simple and inexpensive technique. Limnol Oceanogr Methods 15:200–211

Fulthorpe C S, Hoyanagi K, Blum P et al (2011) Expedition 317 report. Proceedings of the IODP 317. Integrated Ocean Drilling Program, 2011. http://publications.iodp.org/proceedings/317/317title.htm

Gaboyer F, Burgaud G, Alain K (2015) Physiological and evolutionary potential of microorganisms from the Canterbury Basin subseafloor, a metagenomic approach. FEMS Microbiol Ecol 91:1–13

Harrison JP, Gheeraert N, Tsigelnitskiy D et al (2013) The limits for life under multiple extremes. Trends Microbiol 21:204–212

Hoehler TM, Jørgensen BB (2013) Microbial life under extreme energy limitation. Nat Rev Microbiol 11:83–94

Inagaki F, Takai K, Hirayama H et al (2003) Distribution and phylogenetic diversity of the subsurface microbial community in a Japanese epithermal gold mine. Extremophiles 7:307–317

Inagaki F, Hinrichs K-U, Kubo Y et al (2015) Exploring deep microbial life in coal-bearing sediment down to 2.5 km below the ocean floor. Science 349:420–424

Jones W, Leigh J, Mayer F et al (1983) *Methanococcus jannaschii* sp-nov, an extremely thermophilic methanogen from a submarine hydrothermal vent. Arch Microbiol 136:254–261

Jørgensen BB, Boetius A (2007) Feast and famine—microbial life in the deep-sea bed. Nat Rev Microbiol 5:770–781

Jørgensen BB, Marshall PG (2016) Slow microbial life in the seabed. Annu Rev Mar Sci 8:311–332

Kallmeyer J, Smith DC, Spivack AJ et al (2008) New cell extraction procedure applied to deep subsurface sediments. Limnol Oceanogr Methods 6:236–245

Kallmeyer J, Pockalny R, Adhikari RR et al (2012) Global distribution of microbial abundance and biomass in subseafloor sediment. Proc Natl Acad Sci USA 109:16213–16216

Lever MA, Alperin MJ, Engelen B et al (2006) Trends in basalt and sediment core contamination during IODP Expedition 301. Geomicrobiol J 23(7):517–530

Lipp JS, Morono Y, Inagaki F et al (2008) Significant contribution of Archaea to extant biomass in marine subsurface sediments. Nature 454:991–994

Liu C-H, Huang X, Xie T-N et al (2016) Exploration of cultivable fungal communities in deep coal-bearing sediments from ∼1.3 to 2.5 km below the ocean floor. Environ Microbiol 2:803–818

Lloyd KG, Edgcomb VP, Molyneaux SJ et al (2005) Effects of dissolved sulfide, pH, and temperature on growth and survival of marine hyperthermophilic archaea. Appl Environ Microbiol 71:6383–6387

Lloyd KG, Schreiber L, Petersen DG et al (2013) Predominant archaea in marine sediments degrade detrital proteins. Nature 496:215–218

Lomstein BA, Langerhuus AT, D'Hondt S et al (2012) Endospore abundance, microbial growth and necromass turnover in deep subseafloor sediment. Nature 484:101–104

Morono Y, Terada T, Masui N et al (2009) Discriminative detection and enumeration of microbial life in marine subsurface sediments. ISME J 3:503–511

Morono Y, Terada T, Nishizawa M et al (2011) Carbon and nitrogen assimilation in deep subseafloor microbial cells. Proc Natl Acad Sci USA 108:18295–11830

Navarri M, Jégou C, Meslet-Cladière L et al (2016) Deep subseafloor fungi as an untapped reservoir of amphipathic antimicrobial compounds. Mar Drugs 14(3):50

Nunoura T, Soffientino B, Blazejak A et al (2009) Subseafloor microbial communities associated with rapid turbidite deposition in the Gulf of Mexico continental slope (IODP Expedition 308). FEMS Microbiol Ecol 69:410–424

Oger PM, Jebbar M (2010) The many ways of coping with pressure. Res Microbiol 161:799–809

Orcutt BN, Bach W, Becker K et al (2010) Colonization of subsurface microbial observatories deployed in young ocean crust. ISME J 5:692–703

Orcutt BN, Sylvan JB, Knab NJ et al (2011) Microbial ecology of the Dark Ocean above, at, and below the Seafloor. Microbiol Mol Biol Rev 75:361–422

Orsi WD, Biddle JF, Edgcomb V (2013a) Deep sequencing of subseafloor eukaryotic rRNA reveals active fungi across marine subsurface provinces. PLoS One 8:e56335

Orsi WD, Edgcomb VP, Christman GD et al (2013b) Gene expression in the deep biosphere. Nature 499:205–208
Pachiadaki MG, Rédou V, Beaudoin DJ et al (2016) Fungal and prokaryotic activities in the marine subsurface biosphere at Peru Margin and Canterbury Basin inferred from RNA-based analyses and microscopy. Front Microbiol 7:846
Parkes R, Cragg B, Wellsbury P (2000) Recent studies on bacterial populations and processes in subseafloor sediments: a review. Hydrogeol J 8:11–28
Parkes RJ, Cragg B, Roussel E et al (2014) A review of prokaryotic populations and processes in subseafloor sediments, including biosphere: geosphere interactions. Mar Geol 352:409–425
Piepenbrink KH, Sundberg EJ (2016) Motility and adhesion through type IV pili in Gram-positive bacteria. Biochem Soc Trans 44(6):1659–1666
Ravikumar S, Williams GP, Shanthy S et al (2007) Effect of heavy metals (Hg and Zn) on the growth and phosphate solubilising activity in halophilic phosphobacteria isolated from Manakudi mangrove. J Environ Biol 28:109–114
Rédou V, Ciobanu MC, Pachiadaki MG et al (2014) In-depth analyses of deep subsurface sediments using 454-pyrosequencing reveals a reservoir of buried fungal communities at record-breaking depths. FEMS Microbiol Ecol 90:908–921
Rédou V, Navarri M, Meslet-Cladière L et al (2015) Species richness and adaptation of marine fungi from deep-subseafloor sediments. Appl Environ Microbiol 81:3571–3583
Reyes C, Lloyd JR, Saltikov CW (2008) Geomicrobiology of iron and arsenic in anoxic sediments. In: Ahuja S (ed) Arsenic contamination of groundwater. Wiley, Hoboken, pp 123–146
Schrenk M, Huber JA, Edwards KJ (2010) Microbial provinces in the subseafloor. Ann Rev Mar Sci 2:279–304
Schulte M, Blake D, Hoehler T et al (2006) Serpentinization and its implications for life on the early Earth and Mars. Astrobiology 6:364–376
Schouten S, Hopmans EC, Damsté JSS (2013) The organic geochemistry of glycerol dialkyl glycerol tetraether lipids: a review. Organic geochemistry 54:19–61
Smith DC, Spivack A, Fisk MR et al (2000) Methods for quantifying potential microbial contamination during deep ocean coring. ODP Technical Note 28
Starnawski P, Bataillon T, Ettema TJG et al (2017) Microbial community assembly and evolution in subseafloor sediment. Proc Natl Acad Sci USA 114(11):2940–2945
Stevenson A, Cray J, Williams J et al (2015) Is there a common water-activity limit for the three domains of life? ISME J 9:1333–1351
Sturt HF, Summons RE, Smith K et al (2004) Intact polar membrane lipids in prokaryotes and sediments deciphered by high-performance liquid chromatography/electrospray ionization multistage mass spectrometry—new biomarkers for biogeochemistry and microbial ecology. Rapid Commun Mass Spectrom 18:617–628
Takai K, Nakamura K, Toki T et al (2008) Cell proliferation at 122°C and isotopically heavy CH_4 production by a hyperthermophilic methanogen under high-pressure cultivation. Proc Natl Acad Sci USA 105:10949–10954
Teske A, Sørensen KB (2008) Uncultured archaea in deep marine subsurface sediments: have we caught them all? ISME J 2:3–18
Turcotte DL, Schubert G (2002) Geodynamics, 2nd edn. Cambridge University Press, Cambridge
Vreeland RH, Rosenzweig WD, Powers DW (2000) Isolation of a 250 million-year-old halotolerant bacterium from a primary salt crystal. Nature 407(6806):897–900
Whitman WB, Coleman DC, Wiebe WJ (1998) Prokaryotes: the unseen majority. Proc Natl Acad Sci USA 95:6578–6583
Zhu R, Versteegh GJM, Hinrichs K-U (2016) Detection of microbial biomass in subseafloor sediment by pyrolysis–GC/MS. J Anal Appl Pyrolysis 118:175–180