

The Limits of Life and the Biosphere in Earth's Interior

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Fifty years of scientific ocean drilling have shown that microorganisms are widespread deep inside the ocean floor. Microbial populations exist in both organic-matter-rich and nutrient-poor sediments (Kallmeyer et al., 2012; D'Hondt et al., 2015), in sediments that are millions of years old and are buried to over a kilometer depth (Roussel et al., 2008; Ciobanu et al., 2014; Inagaki et al., 2015), and deep inside the basaltic oceanic crust (Orcutt et al., 2011; Lever et al., 2013). In these varied environments, metabolic activity is extraordinarily low (D'Hondt et al., 2009; Hoehler and Jørgensen 2013; Lever et al. 2015a), but microbial cells remain physiologically active (Morono et al., 2011) or survive in their dormant phases (Lomstein et al., 2012). The total amount of subsurface biomass is still being debated (Hinrichs and Inagaki, 2012; Kallmeyer et al., 2012; Parkes et al., 2014), and the factors posing ultimate limits to deep life

and the habitability of Earth remain to be resolved (Figure 1).

Within the ocean floor, geological, physical, chemical, and biological processes interact. Microorganisms can potentially exploit the energy released in these interactions, but they might also be substantially physiologically stressed in this environment. With depth below the seafloor, temperature increases and tectonic influences on heat and fluid flow result in vast temperature variations on local to global scales. Because temperature governs chemical reaction rates, its increase with depth becomes a major stress factor for microorganisms: rates of biomolecule-damaging reactions, such as DNA depurination, polymer hydrolysis, or amino acid racemization increase (Lindahl and Nyberg, 1972; Wolfenden et al., 1998; Steen et al., 2013), and so does the energy demand for biomolecule repair (Röling et al., 2003; Price and Sowers, 2004; Lever et al., 2015a).

Conversely, heating of sedimentary organic matter may also provide microbes with energy through the release of low-molecular weight compounds (Wellsbury et al., 1997; Horsfield et al., 2006). These temperature effects deserve attention as an estimated ~52% of Earth's marine sediment volume resides at temperatures >40°C, including ~25% at >80°C (LaRowe et al., 2017).

In deep, energy-limited subsurface sediments, the upper temperature limit of life is expected to be lower than in surface hydrothermal habitats (Inagaki et al., 2015; Lever et al., 2015a; Møller et al., 2018), where abundant geothermally produced electron donors and seawater-derived electron acceptors provide energy for hydrothermal vent organisms to thrive. Indeed, pure cultures of certain hydrothermal vent archaea can be maintained at ~120°C under elevated pressure in the laboratory (Kashefi and Lovly, 2003; Takai et al., 2008), and several groups of Archaea and Bacteria from shallow seafloor environments thrive at temperatures of 80°–105°C both in the laboratory and in the field (e.g., Burggraf et al. 1990; Jørgensen et al., 1990, 1992; Lloyd et al., 2005; Edgcomb et al., 2007; Teske et al., 2009). By contrast, so far all attempts to isolate high-temperature-adapted microorganisms from deep subsurface sediments have failed. While the absence of microbial activity and viable microbial populations in deep oil reservoirs at temperatures above ~80°C (Wilhelms et al., 2001; Head et al., 2003) is consistent with known thermal limits

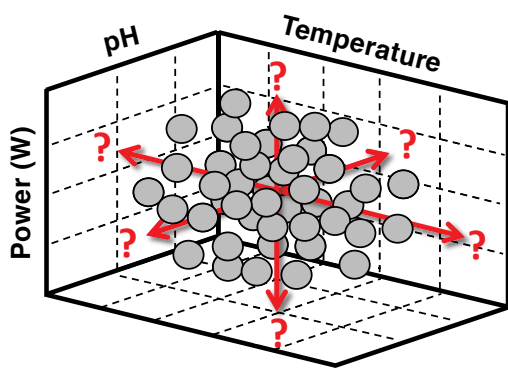


FIGURE 1. Concept sketch illustrating the niches of individual microbial strains (gray circles) with respect to power requirement (in Watts), pH range, and temperature range. The question marks indicate the currently unknown limits of microbial life in subsurface environments. For illustrational purposes, equal niche sizes among different microorganisms are assumed. In actuality, individual strains of subsurface microorganisms may differ greatly in cell-specific power, pH, and temperature ranges that allow

them to thrive or survive. Furthermore, power requirements are likely to vary more systematically with pH and temperature than shown (i.e., subsurface microorganisms living at neutral pH and low temperature may generally have lower power requirements than counterparts living at extreme pH or temperature).

of microbial hydrocarbon degradation (Rueter et al., 1994; Holler et al., 2011; Kellermann et al., 2012; Laso-Perez et al., 2016; McKay et al., 2016), microbiological and geochemical investigations indicate even lower temperature maxima (~60°C) in deeply buried sediments (to ~2.5 km below seafloor; Inagaki et al., 2015).

Chemical disequilibria between seawater and crustal rocks (i.e., predominantly basalts) offer opportunities to exploit energy (e.g., Bach and Edwards, 2003), but the distribution and limits of life in subsurface oceanic crust are even less understood than they are in sediments. Microbes in crustal rocks are difficult to sample with scientific ocean drilling because they are easily contaminated with microbes from drilling fluids. Sealed CORK borehole observatories, in which disturbances caused by drilling disappear and equilibrated fluid compositions similar to those in oceanic crust get

re-established within a few years (Wheat et al., 2010), are an important alternative to acquiring microbial samples from cores. CORKs provide access to fluid samples and free-living cells, and they can be used for colonization experiments (Cowen et al., 2003; Orcutt et al., 2011; Jungbluth et al., 2013).

The still-limited data from rocks and CORKs show large location-specific microbiological diversity, with only minor phylogenetic overlaps between free-living and attached cells (Biddle et al., 2014), and between microbes in crustal rocks and those inhabiting overlying sediments or seawater (Meyer et al., 2016; Labonté et al., 2017; Nigro et al., 2012). Moreover, microbial communities near cold recharge zones (Jørgensen and Zhao, 2016; Tully et al., 2018) differ distinctly from warm locations tens of kilometers downstream from recharge areas (Orcutt et al., 2010; Lever et al., 2013;

Robador et al., 2015). Yet, the factors controlling and limiting life in these hard rock environments are far from understood, and thus subsurface oceanic crust remains a major frontier of deep biosphere research.

Ongoing analyses of samples collected by the International Ocean Discovery Program (IODP) promise to provide novel insights into some of these questions, because the challenges associated with accurate temperature measurement (Yanagawa et al., 2017), rigorous contamination control (e.g., Fisk et al., 2003; Lever et al., 2006, 2013; Santelli et al., 2010; Jørgensen and Zhao, 2016), and sensitive and reliable quantification of microbial populations and activity (Kallmeyer et al., 2008; Morono et al., 2013, 2014; Lever et al., 2015b; Glombitza et al., 2016; Ijiri et al., 2018) have been recognized and successfully addressed (Figure 2). Recent drilling into

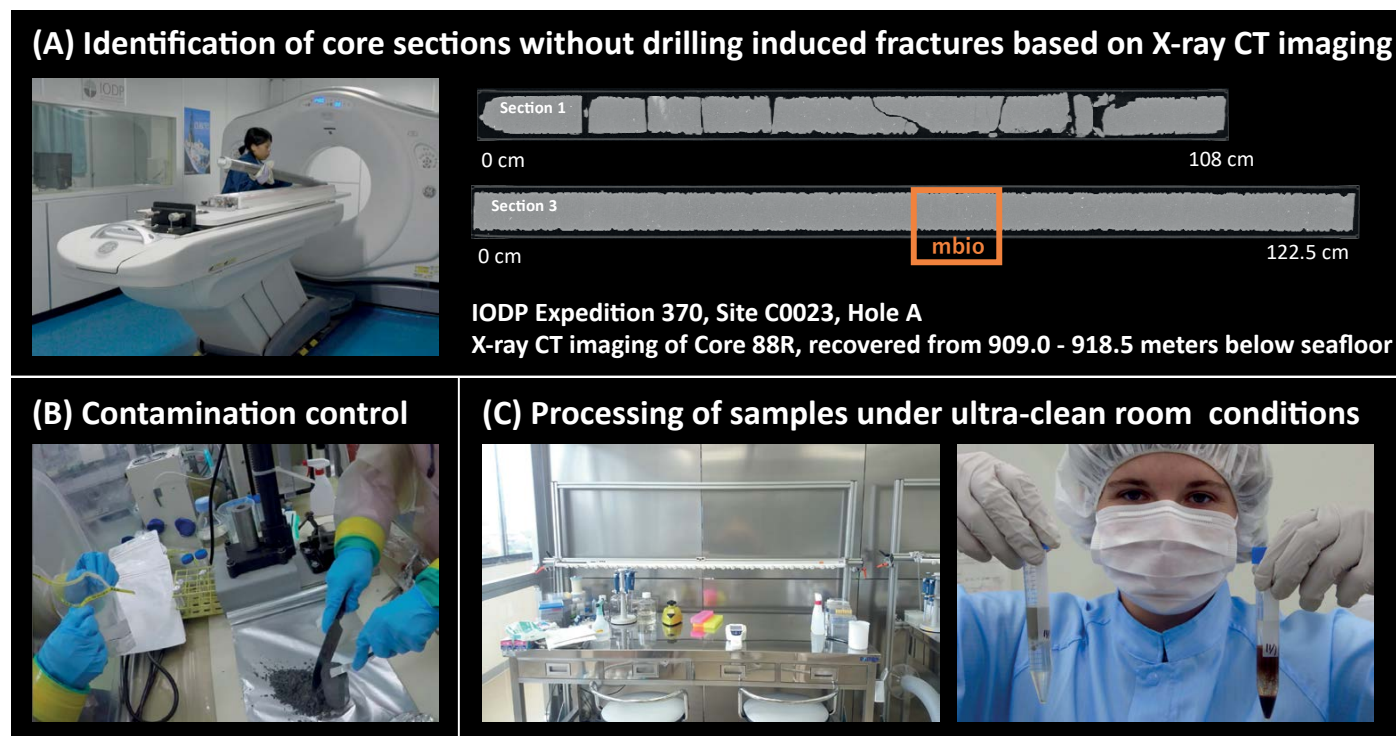



FIGURE 2. State-of-the-art contamination control for microbiological sampling from sediment cores during IODP Expedition 370. (A) On board the Japanese riser drilling vessel *Chikyu*, sediment cores were examined by X-ray computed tomography to identify lithological features and fractures induced in the course of drilling. Using the images, samples for microbiological investigations were selected from undisturbed core sections. The example shows the location of the “mbio” sample taken for cell counting. *Photo credit: Dan Brinkhuis, SCIENCEMEDIA.NL, X-ray images from <http://sio7.jamstec.go.jp/xray-ct/370/C0023A/>* (B) In order to avoid the intrusion of drilling fluid into the inner part of the “mbio” sample, the outer part was carefully removed with sterile tools inside an anaerobic chamber. (C) To avoid introduction of airborne contaminants into the sample, all further processing was conducted in an absolutely dust-free ultra-clean room laboratory at the Kochi Core Center/JAMSTEC. More detailed information is given in the Expedition Report (Heuer et al., 2016).

energy-poor subsurface sediment of the Nankai Trough subduction zone off Japan (IODP Expedition 370) and an upcoming expedition to energy-rich subsurface sediments of the Guaymas Basin in the Gulf of California (IODP Expedition 385, scheduled for 2019) will provide further constraints on the limits of life in subsurface sedimentary environments as a function of energy supply and temperature-driven energy demand. Recent expeditions to the ultramafic Atlantis Massif on the Mid-Atlantic Ridge (IODP Expedition 357) and serpentinite mud volcanoes of the Mariana subduction zone (IODP Expedition 366) will offer new knowledge on the potential for deep water-rock reactions to fuel microbial life. Both of these locations also offer the opportunity to investigate the importance of variables other than temperature, for example, extreme pH, on the distribution of subsurface microbial life. 

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