

plants used as biopesticides, data on microbial biopesticides (bacteria, fungi, virus and protozoa) and predatory insects, sources of liquid resins (*Copaifera longdortii*, *Hardwickia pinnata* and *Dipterocarpus turbinatus*) which can be used as biodiesel,

petrocrops, sources of ethanol and a large number of plants yielding non-edible oils as well as production of biodiesel by methylating the oils, were exhibited. Sources of natural dyes (40) and more than 150 medicinal plants also were presented.

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## RESEARCH NEWS

### Microbial diversity: No limits?

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Though invisible to unaided human eye, prokaryotes are omnipresent and outnumber eukaryotic cells on the earth by several orders of magnitude. They catalyse unique and indispensable transformations in the biogeochemical cycles in the biosphere, produce important components of the earth's atmosphere, and represent a large portion of life's genetic diversity. However, till recently, a vast majority of them escaped the scrutiny of scientists' probing eyes due to the inherent limitations of the methods used to study them. Developments in molecular biology have enabled us to get a glimpse into the lives of microbes recalcitrant to growth in the laboratory. The estimated number of prokaryotic species on earth is thought to be more than  $10^5$  and their total number  $4-6 \times 10^{30}$ . These estimates include sites like marine and aquatic waters, soils, etc. Some recent publications expand the range of known habitats where microbial life thrives.

The first paper deals with marine subsurface communities. Marine sediments overlay two-thirds of the earth's surface and harbour diverse and abundant fauna. It was estimated earlier that of the  $3.8 \times 10^{30}$  prokaryotes calculated to be in the unconsolidated subsurface sediments, 97% or  $3.7 \times 10^{30}$  occur at depths shallower than 600 m. The estimated number of prokaryotes for deeper sediments is only  $0.13 \times 10^{30}$  cells. This value was uncertain because it was based on extrapolation, but it still represents considerable microbial biomass<sup>1</sup>. One would then wonder about their function, metabolism and biogeochemical role at these depths. In other words, what do they eat, how do they re-

spire and what roles do they play? The study by D'Hondt *et al.*<sup>2</sup> provides some clues to these questions. They collected deep-sea sediment cores from equatorial Pacific Ocean off the coast of Peru and measured the number of microbes, potential electron acceptors like sulphates and nitrates, and microbial metabolic products like carbon dioxide, methane, manganese and iron.

In a standard scenario, photosynthesis captures light energy and fixes atmospheric carbon dioxide. This organic carbon is oxidized in the aerobic zone by respiration. As one goes down towards the upper sediments, oxygen is depleted and alternative electron acceptors like nitrate and sulphate that diffuse downward from water column are utilized. This produces a typical profile that is governed basically by the free energy yielded by the oxidants; those yielding greatest free energy are utilized first. The profiles obtained by D'Hondt *et al.*<sup>2</sup> deviated from the expected pattern and gave a strong indication that oxidants which normally diffuse downward from overlaying sea water, have in fact entered the sediments from sub-seafloor sources. There was compelling evidence that sulphates have originated from the brines below the sediment base, and nitrate and oxygen have entered from deep basaltic aquifers beneath the sediment column. This 'upside down' redox profile enables microbes to respire anaerobically and might drive the cycling of iron and manganese.

In another publication, Schippers *et al.*<sup>3</sup> have provided direct quantification of live cells in these sediments. They use an approach that is based on targeting

ribosomal RNA as an indicator for living cells, because only live and metabolically active cells will have functional ribosomes. Dormant or dead cells may maintain intact cell wall and DNA, but RNA would be degraded in inactive cells. They used a highly sensitive molecular technique targeting specifically rRNA as an indicator of living cells, called 'Catalysed reporter deposition-fluorescence *in situ* hybridization' (CARD-FISH), coupled with 'Quantitative real-time polymerase chain reaction' (Q-PCR). Their results confirm the presence of large number ( $>10^5$ ) of active live prokaryotes in these sediments. Bacteria were found to dominate over Archaea. Although D'Hondt *et al.*<sup>2</sup> were able to recover some microbes and characterize them, their role in this metabolism remained questionable, because many of them (*Bacillus*, *Rhizobium*, *Vibrio*, *Paenibacillus*) were close relatives of surface bacteria and thus unlikely to represent authentic deep-sea-surface communities. Further analysis using direct amplification of 16S rRNA genes may provide some information. Some indications already exist that there would be some novel lineages, because one isolate differs from its closest known relative by 14% at the level of 16S rRNA gene sequence. There are also reports of deeply rooted but previously unknown archaeal 16S rRNA sequences.

Another paper deals with deep hypersaline anoxic basins found in Eastern Mediterranean Sea<sup>4</sup>. These have probably resulted from dissolution of subterranean Miocene salt deposits that became exposed to sea water after tectonic activity. Brines in these basins are characterized by an-

oxic conditions, high pressure and almost saturated salt conditions. Each basin was found to be geochemically distinct and it was also presumed that their physical separation might result in independent evolution of microbial life in each. Among these, the Discovery basin was of particular interest due to extremely high Mg(II) concentration (5 M) found in marine environment.

van der Wielen *et al.*<sup>4</sup> undertook a detailed study of these basins to find out their geochemistry and uncultivated microbiota. All the basins, including Discovery showed evidence of *in situ* sulphate reduction, methanogenesis and heterotrophic activity. DAPI staining indicated the microbial counts between  $1.9 \times 10^4$  per ml and  $1.5 \times 10^5$  per ml. 16S rRNA-based community structure analysis, which is a standard procedure to decipher uncultured microbiota, revealed the presence of sequences that represent a new division in Euarchoeota. They have named this division as MSBL-1 (Mediterranean Sea Brine Lakes group 1). The other sequences belonged to known groups like Epsilon proteobacteria, Sphingobacteria and Halobacteria. In contrast, sea water above the basins showed a different community

structure. This is indicative of the fact that a specific microbial community inhabits these brines. Most of the 16S rRNA sequences from Discovery brine were unrelated to phylogenetic groups found in normal sea water and also different from the brine-sea water interface. The community in this brine was found to be different from other brines too. High MgCl<sub>2</sub> concentration might have influenced the evolution of the microbial community here. This observation widens the picture of microbial adaptation to salinity, because most halophilic organisms studied so far can grow under high NaCl concentration, but not under high MgCl<sub>2</sub> concentration.

This fact also has implications on the possibility of extraterrestrial life, because extraterrestrial objects are known to contain brines exposed to evaporation and thus contain high concentration of divalent cations.

Taken together, these studies once again unequivocally emphasize the fact that microbial life can (and does) exist in the conditions that were believed to be not conducive to life. In doing so, they employ novel survival strategies, of which we are unaware today. We have probably also not yet reached the greatest sedimentary

depths that subseafloor organisms can attain. The source of oxidants responsible for 'upside down' redox profile mentioned above remains an enigma. Are they metabolic products of some other microbes? If yes, which ones and what is their effect on global biogeochemical cycle and on mineral, chemical and biological resources? Answers to these questions might change our perspective about life as it exists on earth and might reveal unique metabolic properties acquired by these microbes for survival in these environments.

1. Whitman, W. B., Coleman, D. C. and Wiebe, W. J., *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 6578–6583.
2. D'Hondt, S. *et al.*, *Science*, 2004, **306**, 2216–2221.
3. Schippers, A., Neretin, L. N., Kallmeyer, J., Ferdelman, T. G., Cragg, B. A., Parkes, R. J. and Jorgensen, B. B., *Nature*, 2005, **433**, 861–864.
4. van der Wielen, P. W. *et al.*, *Science*, 2004, **307**, 121–123.

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