Entombed bacteria deep inside the earth

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Scientists' quest for life, past and present, has been confined, so far, to the relatively thin superficial layers of Earth's crust. They have been assuming that no life can exist in the regions other than Earth's ground, air and water, but this view is getting jolted in recent times. The domain of life on our planet appears to extend beyond these regions as living colonies of microbes, in most inhospitable, oxygenless habitats – 3 to 7 km deep within the rocky crust, entombed for millions of years, are increasingly being discovered. In the 1920s, Edson S. Bastin, a geologist at the University of Chicago, and his colleague, Frank E. Greer, a microbiologist, proposed that bacteria and similar organisms called archaea they had collected from certain oil-bearing rocks, more than half a kilometer below, were actually evolved from microbes buried 340 million years ago. They had guessed their presence after they detected hydrogen sulphide and bicarbonate in the waters from the oil horizon and concluded that like some of the sulphate-reducing bacteria on the surface of the Earth, these trapped bacteria must be deriving them by the breakdown of organic compounds in the oil. Fellow scientists who believed that what Bastin and Greer saw were contaminants of surface living organisms, however, received their views with skepticism. All subsequent discoveries of similar life within deep subsurface rocks were dismissed on the same supposition of contamination. Further studies on these subterranean biospheres remained dormant for the next few decades.

During the 1980s, joint researches on these entombed microorganisms were resumed, primarily due to government concern that they may spread into ground water the hazardous and toxic nuclear wastes that have been disposed by burial in deep underground repositories. Research projects were soon initiated and deep drilling at a few sites commenced; the need to recover drill cores, uncontaminated by surface living bacterial forms was given importance and this paved the way for the development of special equipment, innovative aseptic techniques and special confirmatory tests by tracer chemicals and DNA methods. Systematic studies by different teams of investigators in North America and Europe soon began reporting discoveries of these bacteria deep underground and beneath the ocean floors (Table 1)[1-3]. At an experimental drilling site in South Carolina, near the Savannah River Nuclear Processing Unit, the team from Oakridge National Laboratories in Tennessee, discovered the isolated existence of archaea and bacterial colonies in rocks recovered from 500 meters depth. T. J. Phelps of Oakridge National Laboratory, T. C. Onstott of Princeton University and others came across several species of bacteria, 2.7 km below the ground in Taylorsville basin in eastern Virginia[2]. The authors found that some of these bacteria (e.g. *Bacillus infernus*), unlike the surface dwellers were anaerobic thermophiles (heat lovers), living in ambient temperatures as high as 75–140°C. They were supposed to have entered the deep domains, eons ago, through migrating pore-water which is believed to have taken as much as 50 million years or more to reach a site like the one at Taylorsville basin, some 3 km below.

These unique forms of life, confined in a restricted space for millions of years deep in the crustal rocks, as isolated colonies with a population ranging between a few hundreds to several millions, appear enigmatic to our conception of life based on organisms living on the surface. They have been found up to 4 km below continental and 7 km below oceanic crusts, their distribution restricted more by the increasing temperatures, availability of nutrients (C, N, and P), and fuel for energy. After several initial decades, when many doubted the identity of these microbes as forms quite distinct from those on the surface, with the advent of advanced and reliable tools providing unambiguous scientific data, the existence of these microorganisms became an established fact. Yet, a few skeptics continued to suspect that surface-living bacteria must have somehow gained entry, for example, through the lubricating oil that is usually pumped in during the drilling operations. However, as Frederickson and Onstott explain, the doubts of the critics could be set aside, since (a) the oil-borne bacteria were aerobic forms (surviving on oxygen), while the deep dwellers were anaerobic; (b) checks, using experimentally introduced special chemicals (tracers) into the drilling fluids, proved that the extremely fine intergranular spaces in the rocks (a mere tenth the size of surface living bacteria) prevented their entry; (c) in their appearance, the deep dwellers had no resemblance to any of the single-celled surface forms like bacteria, fungi, or protozoa; (d) DNA probe tests conducted on the retrieved rock cores, using special fluorescent dyes, had revealed beyond doubt, the patchy existence of these bacterial colonies; (e) presence of biogenic fatty acid chains (phospholipids), the building blocks of cell membranes[2] at these sites, further confirmed the presence of microorganisms.

Researchers, both geologists and microbiologists, have noted that although the habitats of these bacteria are devoid of oxygen and other readily-available vital constituents, they are yet able to derive them, somehow, from their surroundings. Needless to point out, their requirements for these should be quite negligible, as they have an unbelievably low metabolic rate, and they hardly grow and reproduce. Cell division among them is very slow, taking place once in a century, unlike on the surface of Earth where these divisions occur in minutes to hours.

The basic needs required by these subsurface microbes are obtained through a broad range of chemical reactions that are possible in the deep environments. Since different bacteria thrive on energy derived by different chemical reactions, the type of bacteria populating a site depends on the particular reaction taking place at that site. For example, a porous sedimentary layer permitting the transit of groundwater, may contain oxygen-rich zones supporting abiotic types, whereas, in oxygen-poor zones, the possible chemical reactions like manganese reduction ($MnO_2 \rightarrow Mn^{2+}$), or, iron reduction ($Fe^{3+} \rightarrow Fe^{2+}$), or, sulphate reduction ($SO_4^{2-} \rightarrow H_2S$), or, methanogenesis ($CO_2 \rightarrow CH_4$) accordingly dictate the type of bacteria that can populate the region. Stevens and McKinley[3] of Pacific Northwest National Laboratory, Washington, USA, have observed that most subsurface communities are 'ultimately or indirectly dependent on photosynthesis for their energy; they either use remnant organic

Table 1. Some of the reported occurrences of subterranean bacterial colonies

Location	Findings	Investigators
Oil-bearing sedimentary rocks at 600 m depth, USA	Supposed to have evolved from microbes buried during sedimentation, 340 m.y ago. Findings dismissed as contamination from surface	E. S. Bastin and F. E. Greer
Sediments beneath the seafloor	Find of bacterial colonies rejected by scientists of the period as possible contaminants	C. E. Zobell (Scripps Institute of Oceanography)
Hard rock at 500 m depth in South Carolina, Savannah River Nuclear Facility	Diverse communities of bacteria and archaea detected	Subsurface Science Program of Dept. of Energy, USA.
Taylorsville Basin, Eastern Virginia, USA	Microbes found 2.7 km below were supposed to have been trapped during sedimentation some 230 m.y ago	Joint studies by T. J. Phelps, T. C. Onstott and others with Texas Oil Company
Sediments beneath the Pacific Ocean floor	Bacterial colonies found 500 m beneath in the ocean sediments, subsisting on trapped organic matter	John Parks (Bristol University, UK)
Aquifers in granites, south east of Sweden; and in the Canadian Shield	Lithoautotrophic bacteria found in water flowing through fractures at 400 m depth. The microbes are believed to have gained entry via the groundwater sometime after the parent magma cooled and solidified	Karsten Pedersen (University of Goteberg, Sweden) and Lollar and colleagues (USA and Canada).
Gold Mine in South Africa	Thermophilic bacteria found at 3.5 km depth within hard rocks	T. C. Onstott (Princeton University).
Washington State, USA, Columbia Plateau	Lithoautotrophic microbes within aquifers in basaltic and hardened sedimentary rocks found 1.5 km below	T. O. Stevens and J. P. McKinley, (Pacific Northwestern National Laboratory, Washington)
Undersea volcanic vents of mid-ocean ridges.	Abundance of bacterial forms of diverse types	Undersea expeditions by teams from USA and France.
Hanford nuclear facility site, Washington State, USA	Diverse subsurface bacterial colonies	Department of Energy, USA
Cretaceous shale and sandstone, New Mexico, USA	Bacteria found in sandstone and shale formation living on 100 m.y organic material. Microbial activity in shales very reduced owing to restricted pore size. Microorganisms ferment organic matter and carry out sulphate reduction and acetogenesis	L. R. Krumhotz and colleagues (University of Oklahoma and Pacific and Northwest National Laboratory, Washington)

carbon deposited with the sediments or use the dissolved oxygen as a metabolic terminal acceptor'. In their studies, on the forms they came across in the Columbia River basalts, Washington state, energy was derived from hydrogen released during weathering of the minerals by trapped ancient water (believed to be over 30,000 years old). This is a geochemical reaction involving reduction of H_2O to produce H_2 by iron in the ferromagnesian silicates[4,5]. Hydrogen-dependent bacteria deriving it geochemically are unique, although microbial generation of H_2 is a known process in nature. Since the amounts of H_2 (along with methane, CH_4) these authors had come across in the basalts far exceeded microbial generation (by 1000 times), they concluded that it must have been geochemically derived. In fact, they even reproduced this reaction in the laboratory, using chunks of basalts kept sealed in water for a year or more. Similar geochemically-derived H_2 is believed to support microbial colonies observed in granitic aquifers in Sweden[6] and Canada[7]. Apart from geochemical and microbial generation, as alternate sources, H_2 from nearby volcanic vents or diffusion of trapped H_2 from rocks still deeper down, including the mantle, have also been gested, by a few, who doubt that stri water of its H , requires reducing c tions unlikely[2] to be achieved in subsurface regions.

Presence of organic compounds v the basalts, was another unusual fe noticed by Stevens and McKinley in basaltic rocks. These rocks are igneo origin and hence these compound most unlikely to be original rock con ents. However, their presence coul explained when it was later discovere a particular type of deep dwelling totroph (bacterial forms that synth organic compounds, such as proteins, fats and other carbon-rich molecules) that use H_2 for energy and derive carbon from inorganic CO_2 (acetogens), excrete simple organic compounds that other bacteria live upon. Another geomicrobiological mechanism observed by Frederickson and Onstott, in certain sedimentary rocks, is by breakdown (oxidation) of trapped organic matter nearby, and use of the resulting carbon dioxide to derive their energy, a chemical reaction enhanced in the presence of minerals bearing iron and manganese[1].

The several new finds of complex types of subsurface bacterial communities thriving on diverse sources of energy, should now prompt researchers to turn their attention to different areas of unexplored deep horizons, particularly among the sedimentary strata, where such life may be just waiting to be discovered. While, no doubt, the various new finds of subterranean life have, indeed, extended the limits of biosphere beyond the relatively thin veneer of Earth's surface, it is difficult to assess how many of these forms, ever in a state of suspended animation, are actually alive. Frederickson and Onstott feel that the sluggish metabolism of the entombed microbes is what makes it difficult to judge this aspect. Some of them could be revived in the laboratory, by providing the proper conditions and nutrients, but, several others could not be reactivated; the failures, they say, can be due to (i) inability to reproduce the right conditions in the laboratory, or (ii) the fact that these bacteria may be actually dead, their decomposition, however, arrested or slowed in the sterile subsurface conditions, or (iii) these forms could have lost their ability to replicate themselves.

It is no surprise, therefore, that the unique modes of life of these deep dwellers have stirred up many questions and speculations among scientists. Karsten Pedersen of University of Goteberg, Sweden, points out that if these bacteria and archaea can survive in the Earth's inhospitable environment deep below, life on Earth may, perhaps, have had a beginning there, instead of on the surface, especially since our planet's surface during its early history was marked by repeated bombardment by asteroids and meteorites, a scenario adverse to support any life. Also in the absence of an absorbing atmosphere, which our planet developed much later, lethal UV doses from the Sun must have greatly affected their survival on the surface. Life in the forbidding deep domains also raises the possibility of similar buried life in some of the other planets and moons within the solar system, as well as in some of the new ones being reported from other star systems. They could be lurking deep beneath the surface of Mars, a possibility enhanced in the light of recent findings by USA's Pathfinder, about flooding of water that this red planet had once witnessed. It will not be too much of a speculation, therefore, if these waters that may still be coursing through Martian subsurface, carry microbial life, as happened on Earth, aeons ago.

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