Chapter 2

Microbial Life in Deep-sea Sediments
As oceanic crust moves away from spreading centres, it becomes covered with increasingly thick layers of slowly accumulating sediments, which can accumulate to depths of several kilometers. Sediment layers can be much thicker at continental margins, where they accumulate from weathering of continental platforms and accretion of sediment scrapped off of subducting tectonic plates. The study of biodiversity of subsurface microorganisms from the sediments is only just beginning. A case can be made that the subsurface is both the largest portion of Earth’s biosphere and the most poorly catalogued. In most subsurface studies to date, bacterial diversity has been evaluated through cultivation and enumeration of various metabolic functional groups. In most cases, only aerobic heterotrophs were characterized. In recent years, other functional groups have been routinely enumerated in the subsurface, including denitrifying bacteria, dissimilatory metal reducing bacteria, and methanogenic archaea. Relatively few of these organisms have been characterized mostly because of late surge in this field of study and lack of facilities for the studies.

As mentioned in the previous chapter, the deep sea was thought to be devoid of life till mid 19th century. This assumption was refuted in 1860 by the discovery of corals and sponges attached to a transatlantic cable that was hauled up from the seafloor for repair. From 1868 to 1870, Thomson led multiple cruises of HMS *Lightning* and HMS *Porcupine* to dredge the Atlantic seafloor at depths as great as about 4,600 meters. By the end of his project, he had discovered diverse life on much of the ocean floor. Since then, marine biologists have come to accept the existence of life on the seafloor and in the first few meters of deep-sea sediments. The existence of
high-pressure life was first documented by the oceanographic surveys of the HMS Challenger, during the 1870s. This pioneering expedition for the first time made extensive collections of deep-sea biota, with more than 100 dredgings at depths of more than 1 km. These collections revealed an unexpected abundance of life in the abyssal depths and thus started the fantastic journey of human beings to look into the life in deep sea.

As discussed earlier, conditions like low temperature, high pressure, and low nutrient levels combine to make the deep sea a potentially hostile environment. Most of the deep-sea bottom is stable, cold and dark; therefore it is possible that very ancient life forms may be present in a state of suspended animation in the world’s largest refrigerator. In 1968, the research submarine Alvin accidentally filled with seawater and sank to a depth of 1540 m in the North Atlantic, where it remained for about one year. Upon recovery, it was observed that a boxed lunch consisting of bouillon, a bologna sandwich, and apples were all remarkably well preserved. This famous “bologna sandwich experiment” and its successors initiated a fascinating and controversial chapter in modern marine microbiology.

Zobell & Johnson [1949] first coined the term barophile and Zobell & Morita [1957] obtained the first evidence of peizophilic growth in mixed microbial cultures recovered from the deep sea. Yayanos [1979] reported the first isolate of pressure-adapted bacteria in 1979. Subsequently, many psychrophilic peizophiles with various optimal growth pressures have been isolated and characterized physiologically and genetically. Deep-sea hydrothermal vents are interesting sources of novel isolates, many of which were discovered in the course of investigation into the origin of life.
Thermophilic microorganisms have also been examined physiologically under high-pressure conditions. Thus, studies on the effects of pressure on microorganisms have been mainly performed using two types of microorganisms, psychrophilic peizophiles and thermophilic peizophiles.

As subsurface strata may be effectively isolated from each other, as well as from the subsurface, it is possible that microbial diversity may vary as a function of depth. Certainly, the abundance of broad functional groups of microorganisms has been reported to vary according to geochemical controls, such as the availability of electron acceptors.

Although most studies on diversity discuss the number of species, other aspects are at least as important, for example, there is cladistic or phylogenetic diversity, morphological diversity, ecological diversity and genetic diversity. All four are harder to study and to quantify than species diversity, and all are independent of it [Williamson, 1997]. Phylogenetic diversity gives the relationship of cellular organisms in the form of diagrammatic trees. The higher plants, animals and fungi occupy three small, closely related branches. At a molecular level, multicellular organisms may be very uniform, and this also reminds us that any one measure of biodiversity is insufficient. Morphological and ecological diversity are perhaps more relevant to the preservation of biodiversity, but there are no well-defined guidelines available about how either should be measured. Measuring ecological diversity is even harder, but probably nearer to what is needed for conservation decisions. So it is not surprising that quantitative studies of biodiversity have, apart from a nod to phyletic diversity,
been based almost entirely on species count. It is well known that most species have not been described.

High species richness of deep-sea sediments was first given prominence by Howard Sanders at the Woods Hole Oceanographic Institution in the 1960s. With his co-workers, he found that by washing the mud from anchor-dredge samples of the deep-sea ooze off New England through fine screens, a surprisingly large number of clearly differing species were revealed [Sanders et al., 1965]. He focused his attention on the macrobenthos, a size fraction of the sediment-dwelling community of mainly invertebrate animals that are retained on screens with meshes of 0.25-0.5 mm and have an upper size limit, arbitrarily determined by visibility in sea bed photographs, separating them from the megabenthos.

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. One would then wonder about their function, metabolism and biogeochemical role at these depths. In other words, what do they eat, how do they respire and what roles do they play?

Thus the study of microorganisms isolated from the deep sea promises to provide new information about the origin of life and its evolution, contributing to the overall marine biodiversity, which is poorly described so far. The study of these
extremophiles also gives an opportunity to investigate how life processes work at some of the extreme temperatures (both high and low) and pressures of the biosphere.

Marine organisms can be classified according to the marine environments they inhabit. Thus, there are oceanic species and neritic species depending upon whether the organisms are found in offshore or coastal waters, respectively. Similarly, plants or animals that live in association with seafloor are collectively called benthos. The benthos includes attached seaweeds, sessile animals like sponges and barnacles, and those animals that crawl on or burrow into the substrate.

Fig. 2.1 The basic ecological divisions of the ocean. The neritic (inshore) pelagic zone is separated from the oceanic (or offshore) pelagic zone by the edge of the continental shelf, which is generally at about 200 m depth. Benthic habitats are in black font whereas pelagic divisions in blue (figure not to scale)

In spite of the vast majority of the seafloor permanently submerged below tidal levels, relative to the intertidal regions, comparatively less is known about life in the bathyl, abyssal and hadal zones (Fig. 2.1). This is mainly due to their relative inaccessibility. Although it is possible to dive to several thousand metres in submersibles or to employ remote-controlled cameras, the number of hours of direct observations in the deep sea are extremely less. Most of the information on deep-sea ecology comes from indirect inferences based on animals contained in benthic samples obtained from ships. Whatever the method, expense is the limiting factor in the deep-sea research. Compounding this expense problem is the fact that animal life is just not very abundant in many deep-sea areas, so that it is desirable to have large numbers of samples. But now with new techniques for collection and observation, combined with accumulating numbers of analyzed deep-sea samples, assessment of benthic life in deeper water, is improving.

Most animal phyla are represented in this dark environment of low temperatures, high pressures and predominantly soft substrates of the deep sea. Some deep-sea residents have a cosmopolitan distribution and are found in all the major oceans; other species are restricted to relatively small areas. In general, species become more limited in geographic range as water depth increases. Only about 20 % of the species present below 2000 m in the Atlantic Ocean are also found in the Pacific or Indian Oceans. Many species found in areas deeper than 6000 m are endemic to the hadal region, and many are restricted to a particular trench.
The benthic animals are separated into infaunal and epifaunal species, depending upon whether they live within sediments or on the surface of the seafloor respectively.

The benthic animals can also be separated in different categories based on the size (relative to the mesh size of sieves used to separate animals from sediments) as follows—

Macrofauna (or macrobenthos) are those animals that are retained by a 1.0 mm mesh sieve. These are the largest benthic animals, including starfish, mussels, most clams, corals, etc.

Meiofauna (or meiobenthos) are those animals retained by a 0.1 to 1.0 mm mesh sieve. These are small animals commonly found in sand or mud. The group includes very small molluscs, tiny worms, several small crustacean groups (including benthic copepods), as well as less familiar invertebrates.

Microfauna (or microbenthos) are those animals that are smaller than 0.1 mm in dimension. This smallest size category is largely made up of protozoans, especially ciliates.

Bacterial abundance in deep sea sediments exceeds 100000 cells/cm$^3$, even at a depth of few kilometers below the seafloor [Raghukumar et al., 2001]. Bacteria play a key role in all major biogeochemical cycling processes in deep-sea sediments, where they contribute up to 90% to the benthic biomass [Pfannkuche, 1992].

Apart from bacteria, recognition of viral influence on pelagic processes has in the past few years led to an increased focus on the role of viruses in benthic environments [Danovaro et al., 2001; Hewson et al., 2001; Middelboe et al., 2003;
Mei & Danovaro, 2004; Breitbart et al., 2004]. Such studies have verified that viruses are abundant, diverse and dynamic members of benthic communities. There are $10^7$–$10^9$ viruses cm$^3$ of surface sediment, which is 10–100 times higher than densities usually found in the overlying water column of the same area [Middelboe et al., 2003], and their abundance and activity have been shown to be positively correlated with benthic microbial activity [Middelboe et al., 2003; Glud & Middelboe, 2004]. Due to such high numbers, viruses can therefore potentially affect the bacterial population and biogeochemical processes in the marine sediments.

In the next chapter, we will see the distribution and occurrence of fungi in the marine environment.