CHAPTER 1

INTRODUCTION
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Ocean realm is an abode of unique and interesting biotopes which includes continental margins, coral reefs, hydrothermal vents, oxygen minimum zones, methane seeps, deep sea sediments, sea ice and the surfaces of animals, plants, marine snow and inanimate objects. These biotopes gain more importance because the oceans cover more than 70% of earth's surface. Oxygen Minimum Zones (OMZs) are large volumes of oxygen-deprived waters at intermediate depths in the eastern boundary of tropical oceans where dissolved oxygen concentration is as low as 0.5 mL$^{-1}$ (Stramma et al., 2008) (Fig.1).

Oxygen depletion is widespread in the world oceans occurring as permanent, seasonal and episodic features (Kamykowski and Zentara, 1990) (Fig. 2). The OMZs' area and volume have been estimated as ca 8% of the global ocean (Paulmier and Ruiz-Pino, 2009). Of the total OMZ area, 59% occurs in Indian Ocean (Arabian Sea and Bay of Bengal), 31% in the eastern Pacific Ocean and 10% in the southeastern Atlantic Ocean (Helly and Levin, 2004).
In modern ocean, OMZs are potential traces of Precambrian ocean where reduced chemical anomalies were prevalent and was the abode of archaea. All OMZs exhibit a similar oxygen profile, but the oxygen levels, thickness of the zone and depth of occurrence vary regionally. Most of the studies on the OMZs have been focused on the geochemistry and paleoclimatology due to their occurrence in or near the vicinity of hydrocarbon-rich regions. Molecular oxygen, due to its positive redox potential, is one of the most important reactants in the biogeochemical cycles. The first global study on denitrification of OMZ was provided by Kamykowski and Zentara (1990). However, recently, an unknown process - the anaerobic oxidation of ammonia (anammox) using nitrate in the ocean has been observed first in the sediment and then in the water column of OMZ by Kuypers et al., (2005). These are also regions of ocean acidification that are marked by the presence of reduced chemical species. This region is the key to understand the present unbalanced nitrogen cycle and oceans' role on atmospheric green house gas control. Also, their expansion as the global climate changes and consequently the drastic impact this may have on the global biogeochemical cycles make these zones ecologically significant (Stramma et al., 2008).
Recently, there is an increased interest both in biological and ecosystem studies of OMZ. Since they are not broad continuous habitats OMZ taxa are not global in distribution, but function as isolated habitats with a high degree of endemism. Two taxa viz. ampeliscid amphipods and lucinid bivalves are widespread within the eastern Pacific and in the Arabian Sea (AS). In most of the OMZ regions studied, though reduced macro-faunal species richness is seen, extraordinarily high dominance has been reported (Levin et al., 2001). Thus, OMZs support many characteristics species which remain undescribed and need to be further explored. Hence, detailed diversity studies have to be conducted for metazoans, meiofauna, megafuana, fishes and microbes within OMZs.

One of the earliest microbiological studies in the OMZ was initiated by the discovery of large, free-living sulphur oxidizing bacteria reported as *Thioploca* by Gallardo (1977) in the eastern south Pacific sediments. This was followed by studies on the diversity, structure and behaviour of *Thioploca* in response to varying concentrations of sulphide and nitrate (Maier and Gallardo, 1984; Fossing et al., 1995; Otte et al., 1999). Studies on bacterial sulphate reduction regulation, hydrogen sulphide fluxes and ammonium cycling rates have been carried out in the OMZ water column (Bailey, 1991; Bruchert et al., 2003; Kuypers et al., 2005; Molina et al., 2007). In the OMZs, eubacteria participate in various biogeochemical cycles such as the nitrogen, sulphur and carbon cycles.

The Arabian Sea Oxygen Minimum Zone (ASOMZ) was discovered during the John Murray Expedition (1933-34) aboard RV Mabahiss (Sewell, 1935). However, the results of this study were never published and a thorough understanding of the ASOMZ did not occur until the International Indian Ocean Expedition (IIOE) during 1959-65 (Gage et al., 2000). The open ocean deep water OMZ in the AS occurs permanently between 150-1500 m (Wyrtki, 1971; von Stackelberg, 1972). The zone is formed of a column of water depleted of oxygen that is >750 m thick and extends to an area of 25,00,000 km² (Paulmier and Ruiz-Pino, 2009). This oxygen minimum layer impinges on the continental slope, subjecting sea floor to
permanent hypoxia that persists over thousands of years in the oceans (Wyrtki, 1962; Kamykowski and Zentara, 1990; Helly and Levin, 2004). The rain of detritus from the euphotic layers gets accumulated on the western continental margin of India and as a consequence the sediment gets enriched in organic carbon (>4%) (Paropkari et al., 1992, 1993). This region where oxygen minimum zone impinges on the sea floor creates a strong gradient in dissolved oxygen concentrations and serves as a specialized habitat for the organisms.

Studies on the ASOMZ were conducted during II0E (Gage et al., 2000). Later, the sub-oxic conditions as well as biological and chemical consequences of this phenomenon in northern AS were documented (Paropakari et al., 1992; van der Weijden et al., 1999; Morrison et al., 1999; Naqvi et al., 2000).

The availability of oxygen has a tremendous impact not only on the redox potential of the environment, but also on the energetics of the organisms (Brune et al., 2000). Most of the biomass and biogeochemical activity occurring therein have been attributed to the marine prokaryotes, which are being considered as the major primary producers and heterotrophic consumers in these systems (Fenchel, 1988; Fuhrman et al., 1993). However, microbiological studies in this region have been sporadic. In the ASOMZ, bacterioplankton have been identified by molecular methods and the sequences have been grouped into clades SAR 11 and SAR 406, followed by sulphate reducing bacteria (Desulphosarcina, Desulphofrigus) and sulphide oxidizing bacteria (Fuchs et al., 2005). Jayakumar et al., (2004) observed the highest diversity of nitrite reductase genes (nirS) in sites of active denitrification compared to regions with undetectable nitrite concentrations, thus linking functional diversity and ecosystem chemistry. The oxygen depleted bottom water significantly affects the characteristics of the sediment pore water due to their close contact (Hermelin, 1992). The bacterial diversity studies pertaining to the ASOMZ sediments are sparse except the report on the presence of bacterium Thioploca in the ASOMZ sediments of the northeastern off Pakistan (Schmaljohann et al., 2001).
Recently, the diversity of culturable and non culturable fungi from ASOMZ water and sediment have been reported (Jebaraj and Raghukumar, 2009; Jebaraj et al., 2010).

Study of bacterial diversity in marine environment is important for understanding their distribution, community structure and thereby the functioning of ecosystem. The ability to measure bacterial diversity is a prerequisite for the systematic study of bacterial biogeography and community assembly. Though culture-dependant methods have been employed to study the diversity earlier, it is now well established that the fraction of bacterial community in any environment that can be detected by these methods is only less than 0.1 to 5%. Therefore, cultivation-independent approaches such as PCR based 16S rRNA clone library analysis and denaturing gradient gel electrophoresis (DGGE) are being used to study the diversity and phylogenetic prediction of bacteria. Despite the important role of OMZs in understanding the primitive marine life and chemistry as well as carbon and nitrogen cycles, very little knowledge is gained on the benthic bacterial diversity of the ASOMZ, except for the recent report by Divya et al., (2010) on the diversity and activity of the culturable heterotrophic bacteria of the ASOMZ sediments.

The examination of benthic bacterial diversity will shed light on the life of bacteria thriving in this region and their role in the functioning of this unique ecosystem. Chandler et al., (1997) have compared the diversity obtained from cultivation-dependant and independent approaches in deep subsurface sediments and concluded that culture based methods cannot account for all organisms in given samples and suggested that a combination of both methods would give a comprehensive assessment of diversity. Therefore, the main focus of this study, which is first of its kind in the AS, is to understand the taxonomical and functional bacterial diversity of the OMZ sediments which have been assessed using cultivation-dependant and independent methods and to elucidate the driving forces in the bacterial community structure in this unique ecosystem.