

Marine microbial diversity and ecology: importance and future perspectives

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The world ocean with a coastline of 312,000 km (193,000 miles) and a volume of $137 \times 10^6 \text{ km}^3$, is the largest ecosystem on earth, and has been used for a variety of purposes by man for millennia. Because of its large volume and vast area, influence of the world ocean on world climate is profound. Microorganisms occur nearly everywhere in nature and occupy an important place in human view of life. Marine microbes represent a potential source for commercially important bioactive compounds and their bioremediation capabilities are also remarkable. They also play a crucial role in decomposition of organic matter and cycling of nutrients. Microbes also serve as food for some bottom-living organisms. Our knowledge of marine microbial diversity has, however, been severely limited by relying on microorganisms that have been cultured.

Keywords: Actinomycetes, bacteria, ecology, fungi, marine microbes, virus.

AMONG the three major habitats of the biosphere, the marine realm which covers 70% of the earth's surface provides the largest inhabitable space for living organisms, particularly microbes. Marine microbes thrive not only in the surface waters of the sea, but also in the lower and abyssal depths from coastal to the offshore regions, and from the general oceanic to the specialized niches like blue waters of coral reefs to black smokers of hot thermal vents at the sea floor¹.

The term 'microorganism' encompasses an extensive and diverse assemblage of organisms, such as bacteria, viruses, protists and fungi which exhibit widely different morphological, ecological and physiological characteristics. The domain Bacteria is characterized by prokaryotic cells with bacterial rRNA and membrane lipids that are ester-linked and are diacyl glycerol diethers, whereas viruses are small particles consisting of genetic material (DNA or RNA), surrounded by a protein coat (some have lipid as well) and 10 to 100 times smaller than most bacteria, with an approximate size range of 20 to 200 nm. The protists are the early cells which evolved a distinct gene containing nucleus and are characterized by usually unicellular reproductive structures, true nuclei and chromosomes, and fungi belong to the domain Eukarya, organisms with membrane-bound nucleus. Even though microorganisms are rarely conspicuous in natural environments, it is estimated that about half the biomass on earth is microbial². Furthermore, microbial life is widely distributed: where there is life on earth, there is microbial life – microorganisms

are found in all ecosystems. The term microbial ecology is thus used to describe the presence and contributions of microbes through their activities to the place where they are found.

Microorganisms, which have been evolving on earth for at least 3.8 Ga (10^9 yrs) out of its 4.6 Ga existence, have provided conditions on the planet that have made it habitable for all other species. It has been questioned frequently whether the diversity of Bacteria and Archaea can possibly be comparable to the enormous diversity of plant and animal life? This question can be dismissed by an examination of the scientific view of biological diversity. While zoologists and botanists marvel at the complex morphological diversity of animals and plants, microbiologists wax about the diverse metabolic capabilities of microorganisms and their divergent physiologic lifestyles. Thus, microbial diversity is one of the difficult areas of biodiversity research³ and unlike animal or plant diversity, microbiologists could do little about it.

Marine microbes are being studied for a couple of decades⁴⁻⁶ and the recent reports of hitherto unknown groups such as SAR11 and picoautotrophs such as *Prochlorococcus* are significant contributions in marine microbial diversity. However, from limited and scattered information of the number of species emerges the importance of microbial taxonomy – an experimentally sophisticated and labour-intensive process. Microbial diversity is vastly understated when the diversity of biological life is based on the number of species known of each group. Not only is the bacterial species concept more typological and less evolutionary than plants and animals, but it is much broader and more inclusive⁷. Thus, study of diversity at the genetic level is necessary to understand the phylogenetic perspec-

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tive, mechanism of degradation, and development of novel strategies of treatment. As for microbial genomics, a decade ago the sequencing of microbial genomes could not get off the ground and the first two microbial genomes sequenced were *Haemophilus influenzae* and *Mycoplasma genitalium*. But, today every microbial genome is sequenced on a special-case basis. A genome comprises the full set of genes that the organism can draw on. It defines not only what genes the organism has but what genes it does not and the genome size is not merely dependent on the microbial size, but also on species.

Assessing microbial biodiversity is a daunting task. Exploration of microbial diversity is clearly a topic of considerable importance and interest. Besides, analysis of microbial biodiversity also helps in isolating and identifying new and potential microorganisms having high specificity for recalcitrant compounds.

Marine ecosystems *vis-à-vis* microorganisms

Mangrove ecosystem

Mangroves are unique inter-tidal ecosystems of the tropics, which support genetically diverse groups of aquatic and terrestrial organisms. This ecosystem is ideally situated at the inter-phase between the terrestrial and marine environment and supports a rich and diverse group of microorganisms.

Bacteria: There are different groups of bacteria which get nourished by detritus and in turn help the mangrove ecosystem in different ways⁸. These bacteria perform various activities in the mangrove ecosystem like photosynthesis⁹, nitrogen fixation¹⁰, methanogenesis¹¹, agarolysis¹², production of antibiotics and enzymes (arylsuphatase, L-glutaminase, chitinase, L-asparaginase, cellulose, protease, phosphatase), etc. which result in the high productivity¹³.

Actinomycetes: The mangrove environment is a potent source for the isolation of antibiotic-producing actinomycetes. Rathna Kala and Chandrika¹⁴ tried out different media for the selective isolation of actinomycetes. Further, Rathna Kala and Chandrika¹⁵, and Sivakumar¹⁶ studied the mangrove-associated antagonistic actinomycetes.

Fungi: With respect to marine fungal diversity, the mangrove is the best-studied habitat and most attention has been devoted to the wood-inhabiting fungi which constitute over 50% of 450 species of obligate marine fungi¹⁷. Mangrove forests generate considerable amount of detritus such as leaf litter, woody debris and inflorescence and hence constitute an ideal environment for many detritus-dependent microbes¹⁸. Substantial fungal populations are, therefore, involved in detritus-processing. Studies

have been conducted on filamentous fungi from the mangrove woody litters^{19–21}. About 150 species are found exclusively on decaying mangrove wood, aerial roots and seedlings¹⁷. The mangrove-inhabiting fungi are categorized as ‘manglicolous fungi’, which have a recent report of fossil record from the west coast of India²².

Coral reefs

Coral reefs are confined to shallow-water ecosystems, largely restricted to the seas between lat. 30°N and 30°S. Coral reef is defined as a magnificent and complex community of marine organisms, which are collectively able to construct, modify or maintain the shore environment through the formation of limy (CaCO₃) skeletons.

Bacteria: Reef sediments are one of the main sinks for nutrients, especially phosphorus and nitrogen which is the result of bacterial action. Coral produces mucus which plays a major role in reef metabolism as an important source of organic material and supports high bacterial activity²³. Coral mucus consists mainly of polysaccharides and protein²⁴, and the mucus released from the corals serves as good growth substrates for bacteria.

Deep sea

Of the total sea surface, only 7–8% is coastal area and the rest is deep sea, of which again 60% is covered by water of more than 2000 m deep. The deep sea is a unique and extreme environment characterized by high pressure, low temperature, lack of light and variable salinity and oxygen concentration. Though the geographical area of deep sea is vast, our knowledge, understanding and studies about the deep sea microorganisms are meagre. However, it is a good source of novel organisms for microbiologists and biotechnologists²⁵.

Bacteria: Deep sea bacteria were first recovered by Certes²⁶ as a result of the Travaillier and Talisman Expeditions of 1882–83. Certes²⁷ recovered barotolerant (pressure tolerant) bacteria from depths of 5000 m and suggested that such microorganisms may exist in a state of suspended animation. Further, Fischer²⁸ reported a few colonies resulted from samples collected at depths of >1100 m during a trans-Atlantic crossing by a passenger ship in 1886. Thereafter, improvements in the knowledge of deep-sea biology progressed slowly due largely to the sterling efforts of Zobell and co-workers. This team initiated work on the effect of hydrostatic pressure on bacterial activity²⁹. Thereafter, on participation in the Danish Galathea Expedition of 1950–52, Zobell started his pioneering work on deep-sea microbiology. These deep-sea heterotrophic bacteria utilize the abundant organic carbon or dissolved organic matter in a threshold concentration³⁰.

They cleave refractory polymeric substances extracellularly and supply monomeric and oligomeric molecules to either the bacterial metabolism or the dissolved organic matter (DOM) pool, which may further be utilized by other organisms. The deep-sea bacteria are, therefore, efficiently adapted to extreme oligotrophy and the activity is mainly limited by substrate availability³¹.

Actinomycetes: The deep-sea habitat remained untouched for a long time by actinomycetes and species isolated from this area are mostly novel with potent sources of antibiotics. Colquhoun *et al.*³² isolated a large number of mycolata actinomycetes from deep-sea sediments. From the deep sea sediment an actinomycetes strain was isolated by Imada and Okami³³, which produced a substance that was an inhibitor of beta-glucosidase.

Fungi: Investigations on the occurrence and role of fungi in deep-sea sediments have received little attention³⁴. While many species of higher marine fungi have been collected close to the sea shore, knowledge on occurrence of these organisms in deep oceans is meagre³⁵ except the work dealing with fungal species recovered from benthonic sediments³⁶. Kohlmeyer³⁷ recorded the first Ascomycotina from the deep sea by a submersible test unit with wood panel, but it was not confirmed if they were merely produced upon incubation by dormant terrestrial spores, hyphae or other propagules³⁸. Two new genera and species of Ascomycetes and two new Deuteromycetes from 1615 and 5315 m depth were described by Kohlmeyer³⁵. Kohlmeyer and Kohlmeyer³⁹ described five species of indigenous deep-sea filamentous higher fungi: *Abyssomyces hydrozoicus*, *Allescheriella bathygena*, *Bathyascus vermispurus*, *Oceanitis scuticella* and *Periconia abyssa*. Raghukumar and Raghukumar³⁴ isolated barotolerant fungi *Aspergillus ustus* and *Graphium* sp. colonies from the Arabian Sea and Bay of Bengal, India. Germination of spores, growth of mycelium and enzyme production under simulated deep-sea conditions indicate their active involvement in marine habitats. Gautschi *et al.*⁴⁰ isolated *Penicillium* from deep water sediment at 4380 ft and evaluated the cytotoxic activity. There was also a report on the ancient culturable fungi *Aspergillus sydowii* from the sediment of Chagos Trench, Indian Ocean which was expected to be 0.18 million years old⁴¹.

Extreme environments

Any environmental condition that can be perceived as beyond the normal acceptable range is an extreme condition⁴². Extreme environments can be found in many parts of the world and all these environments are colonized by microorganisms adapted to these conditions.

Bacteria: The environment is considered as 'extreme' if the condition is not moderate. Moderate means environ-

ments with pH near neutral, temperature between 20 and 40°C, air pressure 1 atm and adequate levels of available water, nutrients and salts. Many extreme environments are found in the earth and the microorganisms inhabiting such environments are termed as 'extremophiles'. The study of biodiversity on the edge elucidates the relationships between organism and environment, and unravels the mechanisms of adaptation to extreme conditions⁴³.

Fungi: The number of different species in 'extreme' habitats is typically much lower than in conventional habitats, and higher organisms are often absent⁴⁴. In particular, little is known about the fungi living in the extreme environments except the report on the diversity of fungi from the hypersaline Dead Sea environment, which is one of the most extreme environments for microorganisms on the earth⁴⁵⁻⁴⁷. Highly saline conditions in waters and soils exert a strong selective pressure on the biota, favouring the development of halotolerant and halophilic forms. Biodiversity of fungal life drastically decreases with increasing salt concentration⁴⁴.

Marine bacterial diversity

The study of marine bacterial diversity is important in order to understand the community structure and pattern of distribution. Bacteria are generally less than 1–2 µm in size except for the largest bacterium *Epulopiscium fishlesoni*, a Gram-positive species 200–800 µm long, that lives in the gut of a Red Sea fish⁴⁸. In the marine environment, 90% of bacteria are Gram-negative with different characteristics⁴ and the Gram-negative cell wall is better adapted for survival in the marine environment.

Different groups of marine bacteria

Based on the 16S rRNA sequence analysis, the universal tree of life shows that three domains of life exist: Archaea, Bacteria and Eucarya⁴⁹. Archaea includes unusual microorganisms which grow under extreme environments and differs from Bacteria due to lack of peptidoglycan. Both these domains collectively play a significant role in the marine environment. The different physiological groups of these domains are listed in Table 1.

Role of bacteria in the marine environment

The marine environment is characterized by the hostile parameters such as high pressure, salinity, low temperature, absence of light, etc. and marine heterotrophic bacteria have adapted themselves to survive in this environment – they require Na⁺ for growth because it is essential to maintain the osmotic environment for protection of cellular integrity. Oligotrophy is also one more adaptation because of

Table 1. Different physiological groups of marine bacteria

Group	Physiology	Role in marine environment	Example
<i>Archaeobacteria</i> Sulphate-reducing bacteria	Chemoautotrophs, anaerobic, thermophilic and mesophilic.	Contribute over 50% of the carbon turnover of coastal marine sediments; take part in the cycling of sulphur compounds in sea water.	<i>Desulfomonas</i> , <i>Desulfovibrio</i> , <i>Desulfobulbus</i> , <i>Desulfotomaculum</i> and <i>Desulfococcus</i>
Methanogenic bacteria	Chemoautotrophs, strictest anaerobes, utilize a limited number of simple carbon compounds (hydrogen, carbon dioxide, formate, acetate and methanol) as their carbon and energy sources for methanogenesis.	Utilize trimethylamine in the marine environment as substrate and produce methane as an end-product of their energy-generating metabolism.	<i>Methanococcus</i> , <i>Methanosarcina</i> , <i>Methanomicrobium</i> , <i>Methanogenium</i> , <i>Methanoplanus</i> , <i>Methanococcoides</i> and <i>Methanobolus</i>
Halophilic bacteria	Require at least 12–15% NaCl to survive and grow well even at concentrations up to saturation.	Red colonies formed due to high carotenoid content and dominate in high salt environments, such as salterns and salt lakes; regulate the osmotic pressure thereby resisting the denaturing effects of salt in their environment.	<i>Haloarcula</i> , <i>Halobacterium</i> , <i>Haloferax</i> and <i>Halococcus</i>
<i>Eubacteria</i> Luminous bacteria	Produce light by a simple protein-like substance called luciferin in contact with the oxygen molecule; Gram-negative and motile heterotrophic rods.	Bioluminescence in the deep ocean helps the organisms defensively to startle and divert predators (defence), to attract prey (offence) and to camouflage. Luminous bacteria help in cycling of nutrients in the sea and contribute in the nutrition of marine organisms as gut microflora.	<i>Photobacterium leionathi</i> , <i>Photobacterium phosphoreum</i> , <i>Vibrio fischeri</i> and <i>Vibrio harveyi</i>
Nitrifying bacteria	Oxidize either ammonia to nitrite (<i>Nitrosococcus</i>) or nitrite to nitrate (<i>Nitrococcus</i>) and convert nitrogen to a form readily available for other biological processes.	Extremely important process, since positively charged ammonium ions bind to acidic sediment particles, where they become available for biological processes; more abundant in nearshore waters than in offshore regions.	<i>Nitrosococcus</i> , <i>Nitrococcus</i> , etc.

the small amount of available nutrient. However, heterotrophic bacterial action promotes organic degradation, decomposition and mineralization processes in sediments and in the overlying water, and releases dissolved organic and inorganic substances⁵⁰. The mineralization of organic matter, which is derived from primary producers, results in its being recycled, so that these substances are again available for primary producers. Heterotrophic microorganisms are the major agents shaping the organic composition of the ocean. These heterotrophic bacteria comprise the bulk of microbial populations inhabiting the water column of oceans and are responsible for much of the biological transformation of organic matter and production of carbon dioxide⁵¹. Distribution of bacteria depends on changes in water temperature, salinity and other physico-chemical parameters⁵². Bacteria also serve as an important source of food for a variety of marine organisms. Thus, bacteria not only maintain the pristine nature of the environment, but also serve as biological mediators through their involvement in the biogeochemical processes.

Breakdown of organic matter: Bacteria play a decisive role in the cycle of matter in water, as they are able to breakdown all natural organic compounds into the components from which they have originated⁵³. Decomposition

of protein takes place by proteolytic bacteria, e.g. *Pseudomonas* and other eubacteria. Cellulose is decomposed by cellulolytic bacteria, e.g. *Cytophaga*, *Sporocytophaga*. Chitin, which is synthesized by several marine organisms as extracellular material from algae, cell walls of some chlorophytes⁵⁴, exoskeletons, including molts from copepods and other marine invertebrates⁵⁵ is a structural polysaccharide. However, it is not degraded easily⁵⁶ as there is a report on chitin preservation in fossils⁵⁷. However, this biopolymer is degraded by chitinolytic or chitinoclastic bacteria, e.g. *Bacillus*, *Pseudomonas* and *Vibrio*, by their exoenzyme chitinase. Pectins are also decomposed by numerous bacteria in anaerobic condition, e.g. *Clostridium pectinovorum* and the end-products are pectic acid and methanol.

Mineral cycle: Microorganisms have a great role in the mineral cycle in the marine environment. The element carbon which forms the basis of all organic matter undergoes a constant cycle in nature by various heterotrophic bacteria. Nitrogen, a constituent of the protein, is cycled in aquatic environment by several bacteria. Nitrification is an aerobic process, whereas denitrification is the process used by facultative anaerobic bacteria. Fixation of molecular nitrogen is carried out intracellularly by various bacteria,

e.g. *Azotobacter*, *Clostridium*, etc. Microbial oxidation progresses to sulphate, which represents the terminal step of mineralization of organic sulphur compounds and serves as a source of sulphur for plants. This process is called sulphurification. Sulphate-reducing bacteria are *Desulfovibrio*, *Desulfomonas*, etc.

Sedimentation: Bacteria play an important part in the formation of sediments through their metabolic activities⁵³. Due to bacterial activity in the sediments, the total amount of organic matter gradually diminishes and its composition is changed. As the compounds which are more easily attacked are broken down first, the proportion of substances which decompose with difficulty increases in the deeper layers. Thus, the bacteria colonize suspended particles which change their size and shape and consequently their sedimentation rate. Therefore, the main contribution to the degradation of organic materials and sedimentation in deep sea comes from bacteria⁵⁸.

Marine microorganisms as sources of therapeutic enzymes

Marine microorganisms which are salt-tolerant, provide an interesting alternative for therapeutic purposes. Marine microorganisms have a diverse range of enzymatic activity and are capable of catalysing various biochemical reactions with novel enzymes. Especially, halophilic microorganisms possess many hydrolytic enzymes and are capable of functioning under conditions that lead to precipitation of denaturation of most proteins. Further, it is believed that sea water, which is saline in nature and chemically closer to the human blood plasma, could provide microbial products, in particular the enzymes, that could be safer having no or less toxicity or side effects when used for therapeutic applications to humans⁵⁹.

Screening of marine bacteria for secondary metabolites

Secondary metabolites are compounds with varied and sophisticated chemical structures, produced during the idiophase of microbial growth. One good example of the secondary metabolite is the antibiotic and production of antibiotics from marine bacteria is unique, which has not been observed from terrestrial microorganisms⁶⁰. This was supported by the report of bacteriocidal property of sea water due to the production of antibiotics by marine bacteria⁶¹. The first document on antibiotic-producing marine bacteria was by Rosenfeld and Zobell⁶². Since then, there are several reports of antibiotic-producing marine bacteria⁶³⁻⁶⁵. Thus, the study of antibiotic-producing marine bacteria showing the antagonistic effect against human pathogens is an emerging field of bacteriological research, as strains of pathogenic bacteria recently emerged

are unresponsive or multidrug resistant to the already discovered and used antibiotics⁶⁶.

Molecular approaches to search for discovery

Indeed, only 3000–4000 species of bacteria have been described⁶⁷, though it has been estimated that the number of bacterial species worldwide is close to 3 million⁶⁸. Traditionally, microbial taxonomy has been conducted using a variety of physical and biochemical tests, e.g. morphology, Gram staining, glucose dissimilation, etc.⁶⁹, which allow the grouping of microbial isolates into genera and species. This approach requires laboratory cultivation of microbes in order to separate the various isolates into monocultures. This approach has been used to identify and characterize the culturable marine bacteria. However, typically less than 1% of the bacteria can be cultivated in the laboratory, which are ubiquitous and are presumably 'r' selected species. Because of this limitation, bacterial biodiversity can only be accurately determined using molecular taxonomic tools that obviate the need for laboratory cultivation of isolates. Recent advances in marine microbiology with molecular identification by 16S rDNA sequence have come in the form of discoveries of novel microorganisms and to unexplored genetic diversity in the microbial world. From the genome of the selected isolates, 16S rDNA genes are amplified and the resultant amplification products are subjected to Amplified Ribosomal DNA Restriction Analysis (ARDRA) to identify different polymorphic groups among amplicons. Based on the results of ARDRA analysis, 16S rDNA amplicons of the strains are subjected to DNA sequencing. Then the 16S rDNA sequences obtained are compared directly with sequences in the NCBI database using Basic Local Alignment Search Tool (BLAST). Therefore, the 16S rDNA sequence is a valuable tool for identifying and characterizing bacterial diversity as traditional phenotypic identification sometimes leads to wrong identification⁷⁰.

Approaches to study marine bacteria

Two separate approaches have been used to assess the diversity of heterotrophic bacteria in natural communities. The traditional way of assessing the number of living bacteria is based on their ability to grow in culture media, and being characterized phenotypically and genotypically. A large discrepancy of two to three orders of magnitude between total counts (culture-based CFU count) and viable counts (epifluorescence microscopy) is a normal occurrence in these measurements. Staley and Konopka⁷¹ called this discrepancy 'the great plate count anomaly'. This discrepancy could be the consequence of a variety of environmental requirements and physiological adaptations of marine bacteria⁷² or due to the difficulty in setting up nonselective culture media⁷³. To study microbial ecology

there remains a question: which members of bacterial communities are responsible for the overall activity and what factors control the activity or inactivity of *in situ* populations? To better understand the physiology and ecology of bacterial species, their isolation in pure culture remains an essential step in microbial ecology. For the marine environment, colony forming units provide inadequate description of the relative abundance of bacteria, because traditional cultivation methods do not mimic the real environmental conditions under which the natural population flourishes⁷⁴.

More recent molecular approaches do not require the bacteria to be cultivated; instead, community diversity is assessed by an examination of the extracted nucleic acids, in particular, DNA.

Both the approaches have advantages and disadvantages. The principal advantage of using the classical cultivation approach is that organisms are isolated and therefore available for further study. However, culturability of the bacterial cell is a species-dependent characteristic. Many marine bacterial species have unknown growth requirements and have not yet been cultured. Several media with different compositions have been proposed for isolating new species⁷⁵ and a dilution culture technique has been developed to isolate oligotrophic species which do not grow on nutrient-rich medium⁷⁶. The primary disadvantage of the cultivation approach is that most numerous bacteria from many natural communities cannot be grown in pure culture using the current procedure. In contrast, molecular approaches do not require that. However, there are disadvantages to the molecular approach too, such as difficulty in lysing all bacteria from natural communities, presence of DNA from phages and higher organisms in the community, extraction of DNA from dead bacteria, and difficulty in quantification of important species from the habitat, to mention a few. Furthermore, it is not often possible to determine the physiological type or species from its 16S rDNA sequence by comparing directly with sequences in the NCBI database using BLAST as well as with the sequences available with the Ribosomal Database Project (RDP). For these reasons, it is impossible to determine diversity indices of heterotrophic bacteria accurately in most communities using either cultivation or molecular approaches⁷⁷.

Marine viruses: ecological importance

Viruses are considered the numerically dominant component in all aquatic systems and more common in the marine environment^{68,78}, but little is known about marine viruses. In the past, counting viruses in sea water samples by transmission electron microscopy (TEM) was the standard method. However, this method is tedious, expensive, involves time-consuming preparatory steps and lacks precision⁷⁹. In recent years, stains such as DAPI (4,6-di-

amidino-2-phenylindole) and SYBR Green I have been used for enumeration of virus particles by epifluorescence microscopy⁸⁰. Viral densities may even exceed bacterial densities by 1 to 2 orders of magnitude in sea water⁸¹. There is a report which suggests higher abundance of bacteria than viruses in the deep-sea sediment, where viral growth is dependent upon complex interactions with both biotic and abiotic factors, including bacterial metabolic state and virus supply from the water column⁸⁰. Still due to their abundance in marine environment (10^9 to 10^{10} viruses l^{-1}) and their ability to infect bacteria and phytoplankton, they influence bacterial biodiversity⁸² and phytoplankton primary production in ocean systems⁸³. Viruses may have profound effects on microbial loop dynamics and biogeochemical cycling of organic matter. By lysing bacteria and phytoplankton, viruses may divert carbon away from larger bacteriovores and herbivores and consequently return carbon, which would otherwise be utilized at higher trophic levels to oceanic-dissolved organic carbon pools⁸⁴. In addition, viruses in the ocean also play a key role in the production of dimethyl sulphide (DMS) gas. DMS is the most abundant volatile sulphur compound in sea water and is produced primarily by the enzymatic hydrolysis of DMSP (dimethyl sulphoniopropionate), an algal osmolyte and constitutes about half of the global biogenic sulphur flux to the atmosphere. DMSP produced by phytoplankton is released into the dissolved phase through viral lysis. In terms of potential feedback mechanisms between the plankton and climate, biogenic production of DMS may have an important role. Although a portion of DMS is oxidized by bacteria and by phytochemical reactions within the water column itself, a significant proportion escapes from the sea and is oxidized to sulphur dioxide or to sulphate aerosols⁸⁵. Despite all these factors, biodiversity of virus population is totally unexplored⁸⁴. Viruses may therefore be an important factor influencing global carbon budgets, which in turn have a major impact on climate change.

Marine actinomycetes – a boundary microorganism

Actinomycetes are Gram-positive bacteria with branched filaments. Because of their well-developed morphological and cultural characteristics, actinomycetes have been considered as a group well separated from common bacteria. Among the microorganisms, actinomycetes gained special importance due to their capacity to produce bioactive secondary metabolites and enzymes. Biodiversity of actinomycetes has been studied from different niches of the marine realm, e.g. deep sea, nearshore waters and mangrove environment^{14-16,86-89}. Although earlier occurrences of marine actinomycetes were considered as the terrestrial run-off, molecular^{90,91} and traditional approaches⁹² proved that actinomycetes do occur in the marine realm.

Different genera of marine actinomycetes

As warm and slightly acidic soils tend to harbour a greater diversity of actinomycetes than water⁹³, many studies have been done on the isolation of actinomycetes from marine sediments⁹⁴ and several genera are also reported. Lechevalier and Lechevalier⁹⁵ described 32 genera based on chemical composition but from the marine habitat *Actinomyces*, *Actinopolyspora*, *Micromonospora*, *Micropolyspora*, *Nocardia*, *Rhodococcus*, *Streptomyces*, *Streptosporangium* and *Streptoverticillium* have been reported so far.

Marine actinomycetes as a source of antibiotics

Marine actinomycetes constitute an important and potential source of novel bioactive compounds⁸⁴. Since environmental conditions of the sea are extremely different from terrestrial conditions, they produce different types of antibiotics. Several antibiotics have been isolated from marine actinomycetes by many researchers. The antibiotics are entirely new and unique when compared to those from the terrestrial ones⁹⁶. The specific and potent antifungal antibiotic was isolated from actinomycetes isolated from marine sediment by Meiyang and Zhicheng⁹⁷.

Role of marine actinomycetes in marine environment

Apart from antibiotic production, actinomycetes have a profound role in the marine environment. The degradation and turnover of various materials are a continuous process mediated by the action of a variety of microorganisms. There is a speculation that the increase or decrease of a particular enzyme-producing microorganism may indicate the concentration of natural substrate and conditions of the environment. The cellulolytic activity of marine actinomycetes was described by Chandramohan *et al.*⁹⁸; chitinolytic actinomycetes were reported by Pisano *et al.*⁹⁹. Actinomycetes are also reported to contribute to the breakdown and recycling of organic compounds¹⁰⁰.

Approaches to study marine actinomycetes

Identification and classification are the difficult part in actinomycetes research in traditional systems. Several biochemical tests are performed and with the description of Shirling and Gottlieb¹⁰¹; Nonomura¹⁰² and Tresner *et al.*¹⁰³, identification can be done. But colony isolation is often the most frustrating and time-consuming task, as it involves the examination of morphological characters. Hence, besides the traditional method, advanced method for the identification of actinomycetes through computer software Actinobase, can be followed for genus-level identification, where image files are stored with descrip-

tions of International Streptomyces Project (ISP) and other sources. Apart from these, 16S rRNA studies help determine the phylogenetic relationship and make possible the recognition up to species level using sequence signatures followed by Blast search.

Fungi in the marine environment

Though the existence of fungi in the marine habitat is known from early times, their significance as active participants in marine ecological processes has been overlooked³⁹. Hughes¹⁰⁴ stated that marine fungi cannot be defined strictly only on physiological criteria. They need broad ecological spectrum of definition. He classified them into obligate and facultative forms. Fungi which grow and sporulate exclusively in the marine habitats are considered as obligate, whereas those native in freshwater or terrestrial habitats and are also capable of growing and sporulating in the marine environment are termed facultative. Fungi contribute to the energy flow and productivity of an ecosystem by their presence as a contributory source for meeting the basic requirements of organic carbon of organisms at higher trophic levels.

Starting from Barghoorn and Linder¹⁰⁵ till date, marine fungi have been extensively studied from different substrates. The latest estimate of marine fungi is 1500 species, which excludes those isolated from lichens¹⁰⁶. The obligate marine fungi have about 800 species, including those of Basidiomycotina, Ascomycotina, lichen-forming fungi, Deuteromycotina and Yeasts³⁹, and the total number¹⁰⁷ of filamentous higher marine fungi is about 465.

Biogeography of marine fungi

In spite of the scarcity of data, generalization was made by Kohlmeyer¹⁰⁸. Temperature was the most important parameter among the environmental and biological factors controlling the distribution of marine fungi, although the availability of substrates or hosts, hydrostatic pressure and availability of oxygen are also important factors controlling the worldwide distribution of marine fungi. The vertical distribution of marine fungi appears to be controlled by hydrostatic pressure. Hughes *et al.*¹⁰⁹ proposed five littoral mycogeographic zones based on sea-surface temperature: arctic, temperate, subtropical, tropical and Antarctic.

Importance of marine fungi

In the marine environment, endolithic fungi in calcareous animals are important as producers of carbonate detritus in the deep sea¹¹⁰. Marine fungi occupy an important place in ubiquitous organisms, helping in the decomposition and cycling of nutrients. The fungi and other hetero-

trophs which are active in litter, humus and mineral portions of the soil exploit the energy-rich materials in decomposable organic matter and bring about the release of nutrients locked up in the decaying substances. Fungi contribute to the energy flow and productivity of an ecosystem by their presence as a contributory source for meeting the basic requirements of organic carbon of organisms at higher trophic levels.

Approaches to study marine fungi

Most of the fungal biomass methods consist of vegetative hyphae or conidia that cannot be identified through conventional microscopy¹¹¹. The obvious shortcoming in this protocol is that absence of conidia might be due to the absence of species or to the presence of nonsporulating mycelium. In the initial phases of fungal colonization, between the landing of conidia and their growth into a sporulating colony, newly arrived species will escape detection by traditional microscope-based techniques. Molecular approaches characterize nucleic acids that are present in all stages of the fungal lifecycle, and could circumvent the problems associated with microscopy-based techniques.

Two methods can be useful for study of fungal ecology besides bacterial ecology: terminal restriction fragment length polymorphism (T-RFLP) analysis¹¹² and denaturing gradient gel electrophoresis (DGGE)¹¹³. Although both techniques require expensive equipments, many samples can be processed in a short time and allow profiling of the fungal community richness and evenness¹¹¹.

Statistical approaches to study microbial diversity

Although microbial diversity is one of the difficult areas of biodiversity research, estimation of microbial diversity is required for understanding the biogeography, community assembly and ecological processes¹¹⁴. The number of species has been a traditional measure of biodiversity in ecology and conservation, but the biodiversity of an area is much more than the 'species richness'¹¹⁵. Diversity prediction can be made using statistical approaches that estimate species number from relatively small sample sizes¹¹⁶. Hughes *et al.*¹⁰⁹ noted that both rarefaction and richness estimators which have been applied to microbial datasets, highlighted the utility of nonparametric estimators in predicting and comparing bacterial species number. Rarefaction and richness estimators rely on a species or operational taxonomic unit (OTU) definition. The limitations of this method are that OTUs are counted equivalently despite the fact that some may be highly divergent and phylogenetically unique, whereas others may be closely related and phylogenetically redundant¹¹⁷.

Recently, statistical analyses borrowed from population genetics and systematics have been employed and reviewed for use with microbial datasets to estimate species richness and phylogenetic diversity which do not rely on estimation of the frequency of different sequences¹¹⁷. Reciprocal of Simpson's index ($1/D$), F -statistics (F_{ST}) and phylogenetic grouping of taxa (P tests) may be used as a measure of diversity, which has been widely used for ecological studies¹¹⁶. These combined uses of species richness and diversity estimates provide information that enables deeper understanding of microbial diversity.

Conclusion

The conservation and utilization of biological diversity requires comprehensive knowledge about the species distribution so as to keep the ecological balance in an environment. Recent anthropogenic interventions in marine environment have threatened all lives, including microorganisms. Study of marine microbial biodiversity is of vital importance to the understanding of the different processes of the ocean, which may present potent novel microorganisms for screening of bioactive compounds. As the microbial communities have a complex ecosystem process, biodiversity study explores the distribution and roles in the habitat. Marine microbial diversity can be best studied by a combination of techniques of both conventional and modern approaches for better understanding.

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