

# 1. Marine Microbiology: Facets & Opportunities

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Antoni von Leeuwenhoek's viewing of 'animalcules' under his microscope in the early eighteenth century opened vistas for the science of microbiology. However, when Louis Pasteur a century later summed up his lifetime experience into an everlasting doctrine: "the role of infinitely small is infinitely great", this branch of science was at its infancy. Though it rapidly expanded its repertoire in the nineteenth and twentieth centuries, its marine facet began to reveal in the late 1940s with Claude E. ZoBell coming up with simple devices for aseptic sampling for isolating bacteria from marine environment. From the first isolation of marine bacteria some 125 years ago by Bernard Fischer in 1880s to today, the marine microbiology is a highly evolved, sophisticated and multidisciplinary science. The knowledge-growth in this domain is phenomenal. The vagaries associated with realistic quantification, activity measurements and biodiversity description and analyses have almost vanished. This is an era of precise measurements of biological parameters. With the availability -and modified adaptations- of techniques to marine ecosystem-process-analyses, our understanding of many biological oceanographic aspects is changing in concepts and contents. For example, prior to 1950s bacteria were not thought as agents of biogeochemical cycling in the ocean and, an important text-book on ocean processes, by J. H. Steele: "*Marine Ecological Processes*" published in 1973, did not cover bacterial role in the marine environment. With the development of gadgets and molecular techniques, deciphering the central roles marine microbes play in ocean productivity, biogeochemistry and global climate on one hand, their utility in industrial applications, role in biotechnology revolution and ecosystem restoration-efforts on the other, are perhaps the greatest events.

In the light of multitudes of linkages the marine microbes bear with other biotic and abiotic components, it is important that researches on them become easily understood by as many disciplines of oceanography as possible, in particular by marine biologists and ecologists. Being an inter-disciplinary science, knowledge of at least a few of its facets is necessary for deeper insights into related aspects. With this in fore, I requested my co-researchers at the

National Institute of Oceanography and elsewhere to write for this Book. This Book, "Marine Microbiology: Facets & Opportunities" is, thus, an attempt to bring together "some" facets of marine microbiology as have been made out by my many contemporaries in this part of the world. Various contributed chapters put together in this Book are hoped to provide useful insights on ecology and certain applied aspects of marine microbes in particular of bacteria, fungi and protozoan microplankton. Although this volume may not be very exhaustive and all encompassing, I believe that it has all the ingredients to stimulate the readership to appreciate and adapt the invaluable information that has been provided by these experts.

In his first paragraph in chapter 2, Dr. Chandramohan states as follows: "Now we know that these microbes are present everywhere from the permanently frozen continents, high altitudes (up to 60 km high in the atmosphere), thermal springs, deep-seas to even deep-sea hydrothermal vents. They are the building blocks of any ecosystem and perform varied and all-important tasks that include everything from pathogenesis to fixing atmospheric nitrogen in the soil." These sentences are apt to sum up and to propose that the ubiquitous microbes must be doing something mainly to keep themselves alive and when they so do, there may be consequences that are normally beneficial, sometimes deleterious, to the ecosystem they exist in. Dr Chandramohan has compiled many useful aspects of current research trends in Marine Microbiology. While a great deal of effort has gone in to studying marine microbes by us in India, his 10 points-direction must be appealing to the readers for more than one reason. Essentially, he implies that the research we plan and perform must yield results that are of global significance in relevance and applicability. I believe there will be opportunities for us all to implement at least some, if not most, of his valuable, highly pondered-over suggestions.

From the microbial ecologists' intent, whenever one speaks of microbes these days, the one term that comes to fore probably is bacteria. Role of Bacteria in the classical food-web was perceived as mineralisers-decomposers. Later, with the change in perspective, they were seen as recyclers of organic

material. These realizations have placed bacteria to be significant in trophodynamics of aquatic ecosystems. With their ability of assimilating dissolved organic matter (DOM) in the seawater, heterotrophic bacteria assume central roles in several marine ecological processes. For example, if they did not utilize DOM, the biologically most essential elements such as carbon, nitrogen, silicon, phosphorous, iron would have become recalcitrant and sink to the deep ocean and, over time, be lost from the upper ocean strata and would become unavailable for the all important photosynthetic activity in the euphotic zone. In order that such critical functions are appreciated, I have attempted to describe the various general roles heterotrophic bacteria play in the marine environment (Chapter 3). I have included some of the latest results on bacterial abundance and productivity from Arabian Sea and Bay of Bengal to highlight the role of bacteria in these parts of the world oceans in carbon cycling and microbial loop. Descriptions of carbon and nitrogen cycles in the marine environment are included for a general readership. Linkages to many chapters contributed by my colleagues are drawn so as to be comprehensive.

Ecological importance of sulphate reducing bacteria (SRB) in the marine realms is succinctly described by Dr. Loka Bharathi (Chapter 4). The SRB are among the most interesting microorganisms known and their means of energy production are physiologically unique and ecologically crucial. They are central to cycling of carbon, nitrogen, sulphur and phosphorous and, are important ecologically in re-cycling of organic matter in anoxic zones. With their intrinsic ability to use sulphate -the third major ion in the sea- as the terminal electron acceptor in dissimilatory sulphate reduction process, the SRB are widespread in marine environment. Bacterial associations with SRB lead from syntrophy to synergy and the sum of the total is more than its parts. Alone, SRB could contribute heterotrophically or chemolithotrophically and in combination, bring about great many geochemical changes under different environmental conditions. Reduction of sulfate to sulfide is important since it influences many other metabolic processes. The production of sulfide ions has a profound effect on the availability of trace metals and directly influences their uptake by marine biota. Their hardy nature has made them ecologically competitive even in extreme conditions of temperature, pressure and salinity. These anaerobic SRB not only account for as much as 50% of the total organic carbon turnover but also provide enough sources of electrons for other chemolithotrophic and phototrophic bacteria. From the extensive literature and from her own research,

Dr. Loka Bharathi has also described various ecological (including their role in DMS production), taxonomical and physiological aspects of SRB.

Extreme environments and their inhabitants are fascinating to study. As yet, little is known on processes occurring in salt-marshes -certain examples of extreme ecosystems- and their ecological relevance to the adjacent, usual marine or terrestrial ecosystems. When examined superficially, they appear rather simple, but a closer investigation reveals tremendous diversity of form and process, which make it unwise to extrapolate the knowledge gained at one type of extreme environment. Chapter 5 by Dr. Savita Kerkar is a welcome extension of studies on SRB from extremely saline ecosystems. Her comprehensive review and detailed account of diversity of general microflora, anaerobes in general and SRB in particular would help readers to appreciate the importance of microorganisms in helping life to thrive in such tortuously rigorous environments. Results from her research on SRB from Ribandar, a tropical saltern, demonstrate the extreme abundance of these extremophiles.

In marine environment, the innumerable, ubiquitous and microscopic to macroscopic subdivisions of matter can be called particles that are characterized by properties of mass and observable position in space and time. They are present as solitary items or as aggregates of different types in the processes of turnover, decomposition and sinking flux of both organic and inorganic matter. These "biogeochemical molecules" comprising viruses (0.02 – 0.03  $\mu\text{m}$ ), bacteria (>0.22-1  $\mu\text{m}$ ), picoplankton (<5  $\mu\text{m}$ ), polysaccharide strings and sheets (3 to >100  $\mu\text{m}$ ) and detritus (0.5 – 2500  $\mu\text{m}$ ) as well as inorganic matters such as clay minerals (200-1000  $\mu\text{m}$ ), aluminosilicates (200-1000 nm) and iron colloids (100 – 500 nm) are central to our understanding of water column processes. In principle, most of these particles are exposed to an incredibly diverse variety of bacteria/microbes from many different taxonomic and physiological groups and thus serve as microniches for bacteria to exist in the marine ecosystems. Their importance is still more interesting when these bacteria are seen as two entities viz. particle-associated bacteria (PAB) and the free-living bacteria (FLB). As these two entities perform different roles in the same milieu, they may complement each other's activities or, remain unique. Colonization and growth of associated bacteria are important processes in the degradation and transformation of particles. Dr. Maria-Judith De Souza has described the importance and ecological roles of particle-associated bacteria in Chapter 6. Results from her extensive work on PAB from the tropical Mandovi

Zuari estuarine system are suggesting that the preponderant particle-colonizing bacteria perform better than their counterparts in free-living format.

In their natural environments, microorganisms are exposed to a wide range of physical, chemical and biological stresses. Concerted, post-1980s studies on physiology have led to a general concept on the coping up of microbes in the face of adversities. Since majority of the natural population of microorganisms live in environments including aquatic lakes, oceans and aquifer that are oligotrophic, metabolic adaptations such as spore formation, entering viable but non-culturable state and 'reluctance' to rapidly multiply are indeed helpful for the survival of microbial communities. Ocean is one of the most productive ecosystems, but the biological productivity is affected by many factors including ultra-violet (UV) radiation, oxidative stresses, viral-lysis among others. For example, UV A (320-400nm) affects viability of cells and is responsible for inhibition of up to 50% of the total photosynthesis. UV B (290-320nm) is capable of reducing recruitment and affects trophic-transfer. In addition, it is also possible that free radicals and related chemical species in the environment play an important role in controlling the number and activity of natural population of microorganisms. Information on this subject is very limited currently. It is also known that biological factors such as viruses limit the microbial population in the oceans. Dr Ji-Dong Gu provides in chapter 7 many useful evidences as to what happens to the growth rates, lag time and biomass formation in a select set of environmental bacterial isolates when exposed UV light, hydrogen peroxide and iron. His results are useful to emphasize that we must have a better realization of both short- and long-term ill-effects caused by natural and anthropogenic perturbations on natural communities. He has also discusses the implication of radicals of chemical species and their potential role in understanding the fundamental biology of the natural ecosystem.

The Chapter 8 by Drs. Goes, Balch, Vaughn and Gomes presents results from their ongoing laboratory and field experiments that demonstrate the enormous promise and potential of the flow field fractionation (FFFF) technique for ocean biogeochemical studies. The FFFF is a high-resolution technique that very reliably separates and sizes sub-micron (colloidal) matter into well-defined particle fractions. Although the method has found immense utility in industry and medical applications, its adaptation to oceanography has been very limited. Their carefully planned and executed experiments on the nature and fate of extracellular material released by phytoplankton and the dynamics of phytoplankton-bacterial-viral interactions in seawater have brought

out remarkable newer insights on DOM formation, its dynamics in terms of size ranges within a few hours of particle formation. That bacteria govern their dissolution in a matter of minutes following their abiotic colloidal aggregation is information of primary importance to microbial ecologists. These authors have clearly brought out the reliable potential of FFFF in understanding the microbial ecological dynamics as well as in interpreting the organic carbon fate and formation in the ocean.

De Bary, in 1879, described symbiosis as the living together of any two organisms. Though various kinds of interactions can be distinguished, symbiosis is essentially a continuum from obligate parasitism to very loose association of one organism with another. Dr Shanta Nair, has discussed bacterial-associations, their types and ecological consequences of such associations in greater details (Chapter 9). Her account on the cues one can get from studying and deciphering these interactions using animal-microbe models from the angle of discovery of newer molecules/drugs expounds the underlying principles of animal-microbe interactions. For example, many free-living and sediment-inhabiting marine bacteria have been shown to produce secondary metabolites that display antibacterial properties. These have been widely exploited mainly through cleaving the organismic-interactions and, for the past 50 years, antibiotics have revolutionized medicine by providing cures for formerly life-threatening diseases. As Dr Shanta Nair suggests, despite the immense clinical significance of antibiotics in health care, little is understood on the ecology of the organisms that produce them. Since marine environment harbors a wide range of microbes capable of exhibiting bacteriolytic and antibiotic activity, the better-informed microbial ecologists ought to play a role in exploiting the information gained through studying microbial associations in the sea. The apt description available in this chapter on types of associations using right examples does make one wonder of the enormity of influence the tiny microbes exert in the marine environment for their own and their hosts' survival and biological functioning.

Detritus – 'the dead organic matter in various stages of decay with its associated microorganisms, which is formed from complex physical and chemical interactions mediated by the environmental conditions of the sediment and water column as well as the metabolism of micro- and macroorganisms closely associated with it' - is fed upon by a variety of animals in the marine ecosystem. And, detrital processes are crucial to the marine ecosystem and to detritivores. Dr. Raghukumar's chapter (chapter 10) has experimental evidences to describe the pathways through which organic matter resulting from dead

parts of organisms, including leachates and exudates, molts of animals and, dead plant and animal tissues enter the foodweb through the intervention of decomposer microorganisms which is vital both to remineralization processes, as well as the survival of detritivorous animals. Using results from several of his recent investigations, the major role of fungi in detrital processes in the conversion of less palatable dead organic matter to a more attractive food for the detritivores is explained. Since many commercially important marine animals, including the highly priced shrimps, are detritivores, there is direct human interest in detritus formation. Therefore, Dr. Raghukumar has emphasized on the crucial role of fungi both as decomposers and as part of the 'meal' [to detritivores] in an encompassing sentence: "Contrary to popular belief, fungi do form an important component of detritus in the neretic, as well as the oceanic realms".

For the convenience of attaining an order, I have numbered the chapters of Drs Ramamurthy and Balakrish Nair, Drs Lakshmanaperumalsamy, Thayumanavan and Subashkumar and, Dr. Iddya Karunasagar's group from 11 to 14. These chapters deal with microbial pathogens of human health-concerns, cultured shrimps and, seafood safety. Drs Ramamurthy and Balakrish Nair discuss ecological, pathological and molecular aspects of *Vibrio parahaemolyticus* - a halophilic bacterium first described in 1951- that has become a major public health threat in many countries. Next to toxigenic *Vibrio cholerae*, which was associated with 7 pandemics since 1817, gastroenteritis caused by *V. parahaemolyticus* is a multi-serovar disease. These authors bring out recent epidemiological information and go on to suggest that *V. parahaemolyticus* is acquiring the epithet of becoming pandemic. For example, among hospitalized diarrhoeal patients in Calcutta, India, an abrupt increase in the incidence of *V. parahaemolyticus* was recorded in early 1996 and all the strains were identified as belonging to the serovar O3:K6. In subsequent years, *V. parahaemolyticus* belonging to this serovar caused several outbreaks and sporadic incidences in USA, Canada, Russia, Korea, Thailand, Laos, Bangladesh, Japan, Taiwan and Vietnam. The rapid spread of the O3:K6 strains to different countries after 1996 marked the beginning of the first pandemic of *V. parahaemolyticus*. This was the first report on the occurrence of a single serotype-associated spread of *V. parahaemolyticus*. Research review on this serovar in this chapter highlights that the strains isolated in different countries belong to a single clone and uniformly harbored thermostable direct haemolysin (*tdh*), an important virulence gene of this

pathogen. This chapter deals also with origin and molecular tracking of pandemic strains of *V. parahaemolyticus*.

Drs. Lakshmanaperumalsamy, Thayumanavan and Subashkumar in their chapter (chapter 12), "*Aeromonas hydrophila*: a re-emerging pathogen", provide a review on this important aquatic pathogenic bacteria. The authors have documented several recent works on aeromonad taxonomy, distribution, seafood association, human illnesses, virulence factors, toxins and drug resistance. The last characteristic makes this an important pathogen that has been blamed for many septicaemic, ulcerative and dermal conditions in human beings apart from its known roles as opportunistic and/or obligate pathogen in aquatic ecosystems.

The one major denominator in the success of aquaculture is disease of cultured species. Unfortunately, crowding of reared animals, excessive feeding with an expectation of better growth, low exchange of water to economize on running costs lead to physiological stresses on candidate organisms in confined conditions. Although, aquaculture is among the fastest growing food production sectors in the world, disease outbreaks have caused serious economic losses in several countries. Global loss estimates due to shrimp diseases are around US \$ 3 billion. Thus, diseases caused by microorganisms are most devastating. Drs. Iddya Karunasagar, Indrani Karunasagar and Umesha have contributed an extensive chapter on microbial diseases in shrimp aquaculture (chapter 13). This chapter lists and discusses various microbial diseases of shrimps and strategies for management of such diseases. They also have documented non-microbial and non-pathogenic syndromes. I hope the readers will find it a ready reference for knowing the various afflictions prevalent in shrimp aquaculture.

In chapter 14 on microbial safety of fishery products by Drs Indrani Karunasagar, Iddya Karunasagar and Ammini Parvathi, the reader is brought a different realm of microbes. This succinct review focuses on agents and causes of human illness encountered in seafood and also on issues related to seafood safety and quality management. Including the developing countries like India, fish and fishery products are important food component for a large population. With fish being still the cheap source of protein, there has been an increasing awareness, recently, on nutritional and health benefits of fish consumption. The generally low-fat and high percent of poly-unsaturated fatty-acids (known to reduce the risks of coronary heart diseases), have increased dietary and health significance of seafood consumption. Seafood consumption however, is known for a significant

percentage of food borne diseases. With increased fish consumption and transcontinental exports, there is also an increase in the spread and number of food-borne illnesses associated with fishery products. Karunasagars and Parvathi provide a good deal of information on seafood-borne illnesses and discuss the various microbial (viral, bacterial) and macro-parasitic afflictions associated with sea-foods. They highlight the ugly implications of microbial contamination and their adverse effects on commerce and trade. For example, presence of infectious agents in seafood results in rejection of bulk consignments leading to major economic losses to exporting countries in general, and to seafood processing industries in particular. Harmful effects the microbes can bring about in terms of economic losses and human health can be devastating. Also, as there are projections that by 2030 aquaculture would meet nearly half of our fish consumption, this chapter is useful to realize that maintenance of seafood quality is central to ensuring health-safety from seafood consumption.

Obligate and facultative marine fungi associated with decaying lignocellulose substrates, for example: mangrove wood, leaves, seagrass and algae in the marine environment are important biodegraders and mineralizers. Dr. Chandralata Raghukumar has provided detailed experimental results on lignin-modifying laccase, xylan-degrading xylanase and manganese dependent-peroxidase from fungi isolated diverse marine habitats (chapter 15). In addition, her experimental results demonstrate promising potential for practical applications of these enzymes in various environmental pollution clean-up (bioremediation) efforts. The principle she has exploited is quite simple: as lignin polymer is highly heterogenous, the lignin-degrading enzymes function in a non-specific manner and, as a result, other compounds with aromatic structure (eg. many xenobiotic compounds) are also highly susceptible to degradation by lignin-degrading enzymes. Some of these problematic xenobiotics are bleach plant effluent (BPE) from paper and pulp mills containing chlorolignols; effluents from textile and dye-making units containing synthetic dyes and, highly colored molasses spent wash (MSW) from alcohol distillery wastes containing melanoidin pigments and polycyclic aromatic hydrocarbons (PAHs). She has discussed the important advantages to the environment if the degradative potentials of marine fungi are suitably adapted for industrial effluent-treatment. As primary and secondary treatment of effluents can bring down the oxygen demand only and, not the colors in the effluents that are often very toxic and light limiting, utilizing these natural isolates for enzyme production and application would be ideal.

The lignin-degrading facultative marine fungus *Flavodon flavus* she tested works both at acidic and alkaline pH. It is highly effective in decolorizing BPE, several synthetic polymeric dyes and melanoidin pigments in MSW. Her chapter is informative and offering a ready to follow methodology principles that can aid the reader to venture researches on biotechnology applications.

Ever since the photosynthetic organisms started releasing oxygen in to the atmosphere, the available iron has been converted to its insoluble, ferric forms. As iron is an essential micronutrient required for many biochemical processes within microorganisms, its availability in the utilizable forms and concentrations dictate the productivity processes in the marine environment. To meet the iron requirement in the marine and other natural environments, microorganisms have developed a strategy to acquire iron, which includes synthesis and utilization of siderophores. Dr. Mohandass has compiled the scarce but relevant literature on iron chelators in marine and terrestrial microbiota in chapter 16. The many uses of siderophores and their substituted derivatives in medicine, agriculture, pharmaceuticals, health-care and cosmetics industries are brought forth. Since the importance of siderophores extends beyond their immediate role in microbial physiology, their applications in biotechnology are now beginning to be explored. This chapter will give information on bacterial siderophores, with special emphasis on their role marine productivity, their varied applications by humans and future prospects in biotechnology.

Numerous microscopic studies have described the development of biofilm on surfaces immersed in marine waters. The biofilm development begins with the adsorption of dissolved organic matter followed by the colonization, attachment and growth of microorganisms, and adsorption of particulate matter. It is also believed that bacteria are the most abundant microorganisms to colonize surfaces followed by microalgae, fungi and protozoa during the initial stages of fouling. This implies that the biofilm is rich in labile organic matter and can serve as an excellent nutritional source for the detritus and/or filter feeders. Dr Narayan Bhosle on the basis of his recent studies using a multi-parameter approach based on biological, chemical, biochemical and molecular level has characterized the development of biofilm on metal surfaces. From his extensive experience, he recognizes that this multi-parametric analysis is helpful to better understand the dynamics, nature, nutritional value and sources of biofilm developed on surfaces (Chapter 17). It appears that irrespective of the types of surfaces, the first step in the development of biofilm is the deposition, onto surfaces, of the degraded organic matter derived from biogenic and/

or terrestrial sources. This is generally followed by high abundance of hexoses accompanied by low contribution from pentoses and deoxysugars suggesting the abundance of microalgae between days 5 and 10 following immersion. As the period of immersion lengthens, the relative abundance of ribose and rhamnose increases and that of glucose and fucose decreases suggesting the abundance of bacteria in the biofilm. These analyses of Dr Bhosle are helpful to distinguish that biochemical characterization at the molecular level provide a better picture of biofilm development on surfaces immersed in aquatic environment.

The heterogeneous components of phagotrophic animals in the size range of ca 40 to 1000  $\mu\text{m}$  living in the sediments of marine environment are called meiobenthos. These meiofauna are playing a significant role in the trophic structure and transfer of energy within the benthic ecosystem. Abundance and biodiversity of meiobenthic communities are controlled by physico-chemical and biological characteristics of the sediment. The sediment-nutrient dynamics is closely related to meiofaunal activities and their food comes from a variety of sources. Some feed on benthic micro-algae while others on particles of microbial biomass (bacteria, detritus, dissolved and particulate organic matter). Still others are predatory in nature. Interestingly enough, their grazing varies according to abundance and type of food and, from location to location. Meiobenthos apparently impact microbially-mediated processes including control on their population rather significantly. Their trophic relationship appears to be complex because of a strong coupling between meiofauna and microbial

foodweb. Dr. Ansari has provided an interesting account of meiobenthos-microbe interactions in chapter 18.

On the basis of extensive analyses from tropical (Vellar Estuary, East Coast of India) and subtropical (Hiroshima Bay) regions, Dr. Godhantaraman has presented an account of ecological significance of microzooplankton (chapter 19). Falling within the size range of 20 to 200  $\mu\text{m}$ , these heterotrophic/mixotrophic organisms are being understood, these days, to be pivotal in major oceanographic processes such as organic matter fluxes, microbial loop and nutrient regeneration. With their ability to feed on bacteria and other sub-micron particles in the deep layers, they sustain the fauna that can collect and consume them. These protozoan communities can be termed operators of microbial loop. Understanding their role as bacterivores has been important in linking the carbon flow via heterotrophic food chain. Though they are important phytoplankton grazers, their ability to devour on sub-micron sized bacteria with abilities to assimilate dissolved organic moieties in oligotrophic marine regimes is probably one of the governing events in the export flux. This chapter has useful ecological data on generic diversity, seasonal shifts in their composition and experimental data on their grazing potential.

The two more facets that I feel would have been helpful in this collection are, those that should have provided information on viruses and microbial phylogenetic diversity. However, the last facet is in itself so vast that only a 'small treatment' would have been possible here. Nonetheless, the contributors have directly or indirectly tiled ideas for the future researches including these two aspects. With the disclosure of the existence of such opportunities, I hope that this effort of bringing some facets of Marine Microbiology together would be found useful by the oceanographic fraternity.