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Marine Microbial Ecology: Reminiscence of the status and some suggestions for the way forward

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As the title suggests an attempt has been made to pick out a few aspects of the vast progress that the science of Marine Microbial Ecology has made in the recent years and how we can move forward notwithstanding the challenges.

Microbial ecology is a science that deals with the relationship of microbes with one another and their environment. It covers the three major domains viz Eukarya, Archaea and Bacteria and also the viruses. As microorganisms are ubiquitous, they impact the entire biosphere. They regulate all biogeochemical reactions in the marine environment including carbon and nitrogen fixation and phosphorous and sulfur metabolism. As a consequence of the quantitative magnitude of microbial life, a whopping 5×10^{30} cells, and also by virtue of their biomass alone, they constitute the single largest carbon sink. Even in the total absence of eukaryotes the microbial processes would continue unhindered.

Microbes have dominated biogeochemical processes on Earth for >99% of the history of life. Around half the global net primary productivity is by unicellular phytoplankton in the sea (Falkowski et al. 2000). However in the past few decades man seems to over taken them. Just within the past century (0.00000001% of the history of life), many processes on earth have become dominated by anthropogenic activities. For nearly 75% of the 4 billion year history of life they controlled elemental cycling, organic matter production and turnover and the planetary climate and they continue with the same pace or even more accelerated today (Ducklow, 2008)

Current Status:

Marine microbial communities are central to all processes . From traditional cultivation based experiments to today's molecular analyses based on metagenomics, major strides have been made. Molecular ecology made significant impact by revealing the true scope of microbial diversity and providing genetic markers that could be used to track important species, even in cases where cultures were unavailable. In some cases, metagenomics provided insight into the biochemical adaptations of these organisms. A renaissance in culturing technique led to isolates of many abundant ocean microbes that could then be studied in a laboratory setting. Currently a cluster of approaches that span scales from molecules to ocean basins are being applied to ocean microbial ecosystems, with the result that marine microbiology is becoming a highly integrated science.

The global ocean is an integrated living system where energy and matter transformation are governed by interdependent physical, chemical and biological processes. The fluxes of matter and energy, and the microbes that mediate them are of pivotal importance in the ocean, yet remain poorly understood. With the emergence of the “microbial loop” hypothesis, bacteria are assumed to play major role in food-web models, but were treated as ‘black box’ for the purpose of creating tractable models for geochemical fluxes.. More interactions are becoming explicit. It is now becoming apparent, that trophic systems at the microbial level function as complex consortia.

A combination of genomic approaches is now providing a culture-independent view of the microbial world, revealing a more diverse and dynamic community of microbes than originally anticipated. As methods for determining the diversity of microbial communities become increasingly accessible, a major challenge to microbial ecologists is to link the structure of natural microbial communities with their functions. Culture and culture-independent methods have provided an enhanced appreciation for the microbe’s contribution to the evolution and maintenance of life on Earth,

Armed with ever-improving technologies, microbial ecologists are increasingly in the spotlight. Since the 1970s, molecular techniques, such as whole-DNA hybridization and 16S ribosomal RNA sequencing, have gradually become widely used by microbial ecologists to differentiate species in communities, and, since the advent of whole-genome sequencing, molecular techniques have become more rapid and sophisticated. For microbial ecologists, the benefits of rapid sequencing technologies quickly became apparent. Research moved seamlessly to the collation of inventories of microorganisms

in a sample from any environment using metagenomic analyses. Use of high-throughput genomics and metagenomics in particular has enabled analyses that are breathtaking in scope, including the sequencing of the Sargasso Sea.

Microarrays in Microbial Ecology

Microarrays and high-throughput methods have proven to be a useful to provide targeted DNA sequence information for up to many thousands of specific genetic regions in a single test. A microarray consists of multiple DNA oligonucleotide probes that, under high stringency conditions, hybridize only to specific complementary target nucleic acid sequences . A fluorescent signal indicates the presence and, in many cases, the abundance of genetic regions of interest. Of particular interest to microbial ecologists, phylogenetic microarrays are used for the analysis of phylotypes in a community and functional gene arrays are used for the analysis of functional genes.

A phylogenetic microarray developed by the Andersen laboratory, PhyloChip is able to simultaneously identify many of thousands of taxa present in an environmental sample. The PhyloChip is shown to reveal greater diversity within a community than rRNA gene sequencing due to the placement of the entire gene product on the microarray .

A functional gene array that has been developed by the Zhou laboratory, the GeoChip, is an example of a microarray that dynamically identifies functional activities of multiple members within a community. The recent version of GeoChip contains more than 24,000 50mer oligonucleotide probes and covers more than 10,000 gene sequences in 150 gene categories involved in carbon, nitrogen, sulfur, and phosphorus cycling, metal resistance and reduction, and organic contaminant degradation.

Interesting results have been reported on the adaptive strategies in different depths below sea surface. Genes found in low light adapted but not high light adapted strain include those for nitrite transport and assimilation. This phenotypic expression is dictated by this substrate available below euphotic zone Interestingly MED4 encoded many more genes for high light inducible proteins than its low light adapted strain MIT 9313 which contained more genes associated with photosynthesis. *Synechococcus* genome suggests that it is a more versatile generalists than *Prochlorococcus* with a broader range of functional capabilities. In short, some of these genomic characteristics of these oxygenic marine

phototrophs are consistent with what one could predict from their ecophysiology and the environments which they are distributed.(de Long & Karl 2005)

Research under ecology has been mostly handled by reductionist, that is the most complex system are broken down to simpler parts and attempts have been made to understand these parts in isolation. This has been exemplified in the field of molecular biology where everything is viewed at the level of molecules. However, there is a dawning realization that not all questions get adequately answered by examining at the molecular level. There is still much to be learnt from more traditional whole-ecosystem approaches such as those that focus on pattern of species abundance, food web and community structure. In fact there is now a awareness that all organisms discovered virtually need real counterparts to be able to understand them either at laboratory level or harness them for biotechnological applications. However, in the past, research in microbiology stressed more on the components and now it is evolving towards intra and intercommunity linkages.

With the information explosion, the accent on networking and resource sharing have increased. It is being increasingly possible to incorporate the state of art technique and technology to address pertinent scientific question. Technological advance, ranging from microbial genomics to satellite remote sensing, have furthered our understanding of biological process in the ecosystem and microbial ecology is developing into a more integrated science. It is now becoming evident that the behavior of the whole can be different from the parts. The trend now is geared to understand ecosystems holistically. With large scope in the marine sphere it is beginning to evolve under the umbrella of microbial oceanography.

Ecosystem research is not only now expanding its scope from the micro to the mega world but also links the present to the past-geological past. Ecological research is throwing more light on earth evolution and it links to extra terrestrial life. There is much in common between the *Archbaea* on our planet with the presence of methane and ions in Mars. Europa, one of the moons of Jupiter, appears to be one of the few places that we might find extraterrestrial life in our solar system (the others being Mars and Titan, a moon of Saturn). What makes Europa such an attractive place to look for life is the possibility that it has liquid water, essential for life on earth and is the most probable solvent for life elsewhere, and volcanic activity. Volcanic activity provides some of the heat necessary to keep the water on Europa from freezing and could provide important dissolved chemicals needed by living organisms.

Now the emphasis is on the Arrival of Life on earth rather than its Origin on planet. Consequently, populating the space is becoming more a reality.

Just as interest is growing into the extra terrestrial space, there are teams working on the intra-terrestrials in the deep biosphere. The deep biosphere was largely inaccessible until the 1990s. New techniques made it possible for scientists to make direct observations of this deep biosphere. In particular, oceanographers have developed sub-sea-floor laboratories known as circulation obviolation retrofit kits (CORKs), which seal scientific instruments inside deeply drilled boreholes and make real-time measurements of life in the deepest, darkest realms of the marine subsurface. To date, researchers have mounted only one scientific drilling mission, in 2002, that was wholly dedicated to this biosphere. The existence of a microbial community in the ocean crust has long been hypothesized. Isotopic evidence indicates that a deep biosphere of microbes both scrubs oceanic fluids of organic matter and produces new, yet old, organic carbon *in situ* (Edwards, 2011) Katrina Edwards of the University of southern California and her team are actively chasing the ‘intraterrestrials’— the microbes inside the sediments and the rocks beneath, where not long ago it was thought that life could not exist. She is among a group of scientists who are learning just how resilient and pervasive life is in the deep earth, both under the sea floor and inside the continental crust. Cornell University Professor Emeritus Thomas Gold, who for 20 years directed the Cornell Center for Radiophysics and Space Research, proposes the striking and controversial theory that "a full functioning ecosystem feeding on hydrocarbons, exists deep within the earth, and that a primordial source of hydrocarbons lies even deeper."

Way forward and Challenges

In light of these advances, what are the key areas for improvement that are necessary for microbial ecologists to tackle the big issues that face the planet?

In 2007, after ISME 12 a group of microbiologists chaired by Caroline Harwood spent 3 days deliberating the main questions that face microbial ecologists and the specific requirements that must be met to ensure that the microbiology field continues to meet the important demands that have been placed on it by the need to understand the ecology of two pressing global problems: climate change and disease. Important gaps in our knowledge include a lack of acquaintance on the most populous group of microorganisms, the phages, which have influential roles in horizontal gene transfer, host gene

expression and the structure of microbial communities. Although technology is improving, there is a pressing need for improved methods to make measurements on the microscale as it is this scale that is relevant to microbial activities and interactions. Interactions can have far reaching consequences to global geochemical changes. Although each and every member of a whole community could soon be identified through DNA sequencing, this will require improvements in DNA extraction and sequence assembly.

Furthermore, although it might soon be feasible to sequence the complete genomes of all the members of a microbial community, including bacteria, archaea and viruses, cultivation of most species remains a challenge. Besides microbiologists could try and cultivate mixed communities to more accurately probe community functions. Such communities should also be perturbed to evaluate how communities of organisms, rather than single organisms, respond to change. This would be more meaningful than perpetuating the study of model organisms, which might respond differently to changing environmental cues in the presence of fellow community members.

Such research could be crucial if we are to understand the effects of therapeutics on communities that are hosted within our own bodies and might directly affect health or indeed the effects of changing environmental factors on the microbial communities that keep the biogeochemical cycles of the Earth turning.

It should be noted that even fundamental understanding of marine microbiology and the processes they mediate requires access to the sea usually on board well equipped research vessels 40m in length and ca 10 tons displacement. The funding required would be high and logistic support intense. Even when all this is available the observations at best is only a snap shot of the dynamic processes in a vast ocean and not synoptic. So some laboratories have overcome such problems by using automated measurement. Continuous data loggers for coastal systems which give real time data have already been attempted to include sensors for getting insights into bacterial phylogeny or function.

In 2006, ICoMM (International Census of marine microbes) served as the coordinating body that helped to secure funding from the W. M. Keck Foundation for a 454 DNA pyrosequencing system dedicated to DNA tag sequencing projects. ICoMM's systematic and high-throughput analyses of the sequence variation of rRNA genes have provided a wealth of microbial community sequence data for numerous, poorly understood marine environments. When environmental parameters are recorded together with diversity data, it would be possible to assess the impact of space, time, on microbial communities and

quantify interactions among factors. The integration of laboratory-developed microbiological sensors into observing platforms that track changes at high temporal and spatial resolution will enable autonomous observation of changes in marine microbial diversity in the field (Paul *et al.* 2006; Linda Amaral-Zettler *et al.* 2010)

Such in depth understanding of ecosystems and function would strengthen the field of marine biotechnology. Biotechnology as understood some time back began with the serendipitous discovery of penicillin by Alexander Fleming. Though man has made use of traditional biotechnology and continues to do so in many ways, his demands have been increasing and he tries to speed up nature in many ways. Sometimes it is to coax nature to yield products of interest at a pace much faster and quantity much greater. Consequently this applied aspect should graduate from random and chance finding to more directed and probing for the required results. This is where a linkage to microbial ecology would be useful to address a number of environmental and economic challenges. To give a few examples...Managing the carbon cycle to sequester carbon dioxide and prevent excess methanogenesis is important in reducing global warming. Microbial fuel cells could help in the developing bioenergy. Elucidating marine microbial symbioses would help us to explore novel antimicrobial agents, in the arms race against antibiotic resistance or even to harness a number of bioactive compounds with varied applications.

Another interesting area where biotechnology can connect to microbial diversity would be in the area of conservation. One would wonder if there are problems about endangered species and extinction at microbial level too. The challenges would be to address this aspect and understand which major species are around to be able to appreciate changes in biodiversity that could happen in the current scenario of global warming. Added to this is the idea of promoting natural areas of preserving and maintaining microbes in addition to natural culture collection.

The maintenance of diversity is fundamental to the evolution of biogeographic pattern at different spatial and temporal scales. Generally, the combination of speciation, extinction, and dispersal are proposed to explain the generation of biogeographic pattern.

This field should also be explored to develop new analytical and conceptual tools that would systematically include spatial scales in environmental microbiology studies. In that context, more interactions between microbial ecologists, mathematicians, and statisticians would necessarily be

fostered to address issues specific to the prokaryotes (Ramette and Tiedje, 2007). It would be interesting, for instance, to determine whether the generation of biogeographic patterns in prokaryotes is either deterministic random, or chaotic. Different evolutionary scenarios, attempts to posit that biogeographic patterns could consist of a vast range expansion or regression events resulting from evolutionary and ecological forces at play starting from genotype level. This could reflect the fact that the formation of biogeographic patterns may be a dynamic process of expansion or regression of the habitat range at the genotype level, which may be governed by the complex interplay of the nature of the genes gained, lost, or modified, the extent of dispersal and extinction, and natural selection.

For ecosystems to evolve diversity had to take the centre stage. During the last 2 decades, a large array of molecular approaches has been developed and applied to assess the diversity of bacterioplankton. One main result of the classical molecular approaches, such as community fingerprints and environmental clone libraries, is that the bacterioplankton community contains only a few abundant species and a large number of less abundant to rare members. This is very different to soil bacterial communities, where no abundant species are detected and the total number of species is substantially larger. New approaches, such as metagenomics and pyrosequencing, are able to determine the diversity and abundance of the rare members of the community and the biogeochemical potential of the community. Several new approaches, such as stable isotope probing (SIP) and multi-isotope imaging mass spectrometry (MIMS), have been developed to study structure–function relationships of microbial communities. The combination of biogeochemical stimulation experiments with these functional and/or molecular approaches has the potential to elucidate the role of specific microorganisms concerning specific biogeochemical functions. Any local bacterial community include two major groups. The abundant member which form a core community that carries out the ongoing biogeochemical functions, and the rare members which could form a seedbank that provides the genetic potential to respond to any change in the system. Together, they may be able to mediate the biogeochemical processes in the pelagic ecosystems of the earth.(Hofle et al 2008)

However, in the carbon mass balance the microbial role is still far from fully understood, especially in terms of loss processes. Since bacterial growth (and thereby consumption of DOC and inorganic nutrients) is likely regulated differently in different bacteria, it will be critical to learn about the life strategies of the key bacterial species to achieve a comprehensive understanding of bacterial regulation

of C fluxes. Finally, some processes known to occur in the microbial food web are hardly ever characterized and are not represented in current food web models.

Before the development of molecular techniques, microbiologists were largely reliant on traditional methods of identification and isolation to probe the structures of microbial communities. This approach has not disappeared, and indeed should still be used, together with molecular analyses, to fully understand community function. Analyses of the natural *microbial assemblages* by nucleic acid based methods from genomes to biomes have detected novel microbes with novel functions or novel lineages. These are yet to be brought to culture, and therefore lack formal description. Contemporary microbial oceanography could offer a sea of opportunities for young researchers (Karl, 2007)

Suggested further reading

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