Microbial activity in aquatic systems from cell to biosphere

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ABSTRACT

The field of aquatic microbial ecology is flourishing. At the cell and molecular level, aquatic ecologists are unravelling the taxonomic composition of natural microbial assemblages, and evaluating cell-specific activity *in situ*. At the biosphere level, detection of changes in concentration of atmospheric oxygen has shown a strong seasonal signal of microbial production and respiration in the sea on a hemispheric scale. Future challenges include (but are certainly not limited to): 1) time - understanding temporal variability in microbial activity, particularly with respect to season; 2) temperature - evaluation of how rises in temperature in a warming world will affect microbial activity, and thus system function, in aquatic ecosystems; and 3) taxonomy - extending studies of microbial diversity and of cell-specific activity to additional aquatic habitats and to protists. There is also the exciting possibility that in the not too distant future, microbial life may be discovered on other planetary bodies in our solar system.

Introduction

At the very end of his autobiography, 'Naturalist', Harvard professor Edward O. Wilson wrote, "If I could do it all over again, and relive my vision in the twenty-first century, I would be a microbial ecologist"[51]. This statement, from one of the best regarded scientists in the world, is an indication of the excitement generated by our field. Microbial ecology is still a young science, with important new discoveries to be made. The progress that has already occurred has created general public awareness that there is a lot more to bacteria than 'germs', that microbial activity sustains the healthy functioning of natural systems, and that there is great commercial opportunity in microbes. Here we would like to explore what we perceive to be some of the 'leading edges' of the field of aquatic microbial ecology, in which recent findings are discussed in the context of 'where do we go next?' This is our own perspective of some of the topics on which bright young investigators might be working in the next century.

The field of aquatic microbial ecology is large and complex. It spans studies of microbial organisms in the sea, which occupies over two thirds of the earth's surface; in freshwater – which includes lakes, rivers, hot springs, and groundwater; in aquatic sediments; in hydrothermal vents and methane seeps; and in marine and freshwater ice. While 'microbial ecology' often is thought to imply *bacteria*, in aquatic systems virtually all of the carbon transformations and elemental cycling at the base of food webs are accomplished by *microbes* - algae, heterotrophic protists as well as bacteria. In the classic 'changing paradigm' paper of L.R. Pomeroy [33], one of the major points was the

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importance of nanophytoplankton in marine production and respiration. The role of microbes, including bacterial as well as algal autotrophs, in primary production constitutes a major difference in the structure of aquatic versus terrestrial food webs.

Many of the advances in aquatic microbial ecology in the last 25 years have involved discoveries of new groups of organisms. Initially, these were of new and expanded functional groups. Discovery of the high abundance of pico-autotrophs: coccoid cyanobacteria and < 2 μm sized algae in the sea and in oligotrophic lakes [43], and then of pelagic prochlorophytes in the ocean [7], extended downward the size range of primary producers in aquatic systems. Discovery of food webs based on chemosynthetic sulfide-oxidizing bacteria, growing as mats and as internal symbionts, at hydrothermal vents was of enormous scientific and public interest [21]. Such systems now include methane seeps and methane ice communities on the sea floor, in which food webs may depend on growth of methane oxidizing bacteria [30]. During the same period, the importance of phagotrophic protists, as grazers of picoplankton and of phytoplankton of all sizes [37], and as a food resource for larger zooplankton [42], in both marine and freshwater systems, was firmly established. Finally, viruses, including bacteriophages and viruses that infect algae, have been found in high number in aquatic systems, and are thought to be an important source of mortality for bacteria and for phytoplankton blooms [16,44].

The second wave of discoveries about microbes in aquatic systems has involved the application of molecular genetics to microbial diversity. New, taxonomically distinct, and previously uncultured species, and groups of species of prokaryotes have been identified in marine [11,12,13,17,19], hot spring [47] and lake [31] ecosystems. One of the more interesting findings is that archaeal genomes appear to be widely distributed in marine bacterioplankton [12, 15]. No longer can we view bacterial assemblages as simply a 'black box' of unknown composition. Taxon-specific genetic probes allow us to track the abundance and relative activities of identified taxonomic components of microbial communities *in situ* [17,24]. The real excitement is in not simply knowing 'who is there', but in combining molecular genetic approaches to determining 'who is active, and just what are they doing?' Examples are identifying the presence and expression of specific genes, such as the *nir* gene for dissimilatory nitrite reductase [46], and determining cell-specific abundance of rRNA of specific taxonomic groups of bacteria [24].

Some prospects for future research in aquatic microbial ecology

TIME - temporal scales of microbial activity

Investigations of microbes *in situ* have considered spatial scales from ecosystem level down to nanoscale patchiness [23]. Time scales are equally important. Two examples of temporal scales in microbial ecology will illustrate the need to focus on time as well as spatial variability in microbial processes in future studies.

Diel variability in heterotrophic microbial processes

The idea that if the growth of heterotrophic bacterioplankton in the euphotic zone depends directly on photosynthetic production, then there should be day/night cycles in bacterial activity is not new. However, there have been relatively few investigations of this hypothesis, and past studies have yielded equivocal results. Recently, Gasol et al. [18] reported strong diel cycles in bacterial incorporation of radiolabeled leucine and thymidine

in the oligotrophic Mediterranean Sea, with highest uptake rates during the day, and lowest rates at night. These results are empirical evidence for close coupling between bacterial and phytoplankton production. They also show that for systems with close trophic coupling, the time of day in which samples are collected for bacterioplankton productivity assays does matter. In such systems, short-term (diel) temporal variability is as important a consideration as is small-scale (cm to meters) spatial variability.

Seasonal variability in heterotrophic microbial processes

Newly available data on the seasonal variation in atmospheric oxygen content show a strong seasonal signal in oceanic production and respiration at a hemispheric scale [2,22]. The marine component of the seasonal atmospheric oxygen signal indicates net production of oxygen in the upper water column from late winter to mid-summer, and then net diffusion of oxygen from the atmosphere to the sea during the rest of the year [2,22]. The annual increase in atmospheric oxygen during spring is equivalent to a net community production of about 50 gC/m2/yr, 5-fold greater than measured rates of export flux via sinking particles, and 1/3 of average annual primary production in the ocean [38]. This is a large amount of organic carbon that does not appear to be immediately respired by heterotrophic microbes. This phenomenon indicates a seasonal offset of weeks to months in the annual cycle of production and consumption of organic carbon in the sea. Such an offset is at odds with studies, like that of Gasol et al. [18], which indicate a tight trophic coupling between phytoplankton production and bacterial activity. Elucidating mechanisms that contribute to the presumptive seasonal offset in production/respiration processes in the sea, and likely also in lakes, can be a goal of future research in aquatic microbial ecology.

TEMPERATURE - in the context of both seasonal cycles and of potential effects of global warming on aquatic microbial processes

Temperature has long been recognized as a major controller of microbial activity, and must be an important factor in determining seasonal cycles of microbial heterotrophy at mid to high latitudes. However, there seem to be fewer investigations of this physical control on microbial processes *in situ* than studies of biotic controls, e.g. substrate quantity and quality, grazing, and viral mortality. Present concerns regarding response of natural systems to global warming are renewing interest in temperature effects on aquatic microbial communities. Recent studies have demonstrated striking effects of temperature on bacterial activity in lakes [14] and in marine systems [40,45,49]. Such effects include winter/summer differences in proportional uptake of radiolabeled leucine and thymidine [40,45]. We have found markedly different responses to added substrate by coastal marine bacteria with temperature shifts of 4-8^{o}C up from ambient [6]. Since climate change models predict greater, and earlier, warming in polar environments compared to other regions of the planet [5,26], temperature effect studies in higher latitude aquatic systems would be especially relevant [48].

TAXONOMY/SPECIFIC ACTIVITY - extending molecular approaches in aquatic microbial ecology

Despite the avalanche of publications on molecular diversity of microbes that has appeared in the last decade or so, this area of research is really just beginning to flower. Investigations of prokaryotic taxonomic diversity are being extended to diverse habitats and

associations, for example the deep-sea [13,32], deep aquifers [41], water pockets within ice in Antarctic dry lakes [34], endonuclear symbionts of protists (1), and bioluminescent symbionts of marine fish [20]. Application of molecular techniques to investigate the diversity and distribution of heterotrophic eukaryotes (protists) in aquatic systems is still at a very early stage [25, 36].

The greatest gains will accrue from determining cell-specific activity for selected taxonomic types of microbes. This in turn depends on methods, such as taxon-specific rRNA probes, and probes for specific genes, that are still in development. Studies using general diagnostics for cell-specific activity, e.g. CTC, a fluorogenic indicator of ETS activity, have shown that only a small proportion of bacterial cells in natural aquatic assemblages are highly metabolically active [8,39]. Use of cell-specific stains that detect membrane integrity has shown that variable fractions of suspended bacterial cells have intact cell walls and membranes in both marine and freshwater [9,27,35,50] systems. Bacterioplankton in oligotrophic systems also appear to have very low growth efficiencies, often < 5-10% [3,10]. These results support the idea that in general, most bacterial cells observed in aquatic systems are either dormant (in a state of starvation/survival) or dead [28]. However, we have found that a large fraction (up to 80-90%) of apparently metabolically inactive bacterial cells can be induced to show detectable ETS activity (via the CTC method) within 20 - 30 hours with temperature increase and/or addition of organic substrate [6]. It would be of great interest to determine the relative state of metabolic activity of specific taxonomic components of a bacterial assemblage at any one time, and then to be able to determine what factors control their level of activity, as well as the overall growth efficiencies of the assemblage.

OTHER TOPICS

A number of other research directions could also have been discussed here, for example: chemical communication between microbes; symbiotic associations; importance of microstructure/microniches to microbial processes in aquatic milieu; biofilms; impact of pollution/eutrophication on aquatic microbial processes. Farther out on the research horizon for microbial ecologists is perhaps the most exciting prospect of all - the search for extraterrestrial life, a discipline that the US National Aeronautics and Space Administration has now formally recognized as 'Astrobiology' [4]. If life is found on other planetary bodies in the solar system, it undoubtedly will be primarily, or exclusively, microbial life. Models for such organisms are microbes living in extreme environments on earth: anoxic waters, deep subsurface aquifers, hot springs, hydrothermal vents, and ice, all habitats of interest to aquatic microbiologists [29]. Among those who attended this ISME-8 symposium may be scientists who will be directly involved in the discovery and description of the first known extraterrestrial life forms.

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