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Microbial diversity: the importance of exploration and conservation

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Microbial diversity is fundamental to maintenance and conservation of global genetic resources. As extreme environments are explored, the richness of microbial diversity is increasingly evident. Measures must be taken to estimate, record, and conserve microbial diversity, not only to sustain human health but also to enrich the human condition globally through wise use and conservation of genetic resources of the microbial world.

Keywords: microbial diversity; biodiversity conservation; marine microbial diversity

Introduction

Microorganisms—procaryotes, viruses, viroids, filamentous fungi, yeast, microalgae, and protozoans—comprise the greatest numbers of individual organisms on Earth [37]. At best, however, science has identified and is aware of only a minute fraction of them. Somewhere between 1% and 0.1% of all extant bacterial species have been described [14] and the vast majority of the remaining 4×10^5 to 3×10^6 bacterial species [49] is unknown. Most cannot be cultured using currently available techniques [4,13,55].

About 1% of the approximately 5×10^5 viral species has been described [13], along with 5 to 10% of the approximately 1.5×10^6 species of fungi [43], and 4×10^4 of the approximately 1 to 2×10^5 protozoan species [49].

Viroids have been studied in an agricultural context, with little known about occurrence of viroids in the marine world. Obviously, this is an area ripe for exploration. The protozoa merit attention, and reviews of protozoan diversity [21] provide information on this major grouping of microorganisms.

Yet, the natural habitats that harbor these undiscovered microorganisms are disappearing rapidly. Destruction of tropical forests is estimated at between 16.4 and 20.4×10^6 hectares per year. Only a small percentage of the Earth's temperate forests remains. The amount and quality of the world's wetlands are in decline, and only the deepest parts of the ocean appear to have maintained some degree of pristine quality [33].

Microorganisms, too, are disappearing from the Earth. *Penicilliopsis clavariaeformis*, which lived on a species of *Diospyros* tree in Indonesia, disappeared when the tree species disappeared, as did *Cookeina tricholoma*, due to environmental disturbances in its native Java [8].

Standard policies, such as the US Endangered Species Act (ESA) and the Convention on International Trade in Endangered Species of Fauna and Flora (CITES) protect individual species [19]. But microorganisms, especially those we have not yet discovered, cannot be protected in this way. Habitat protection is a means to protect organisms and their habitats [19], a method that would be more beneficial to protection of microbial biodiversity.

The term biodiversity, according to Erwin [20], is related to the number of species, or species richness, along with 'the richness of activity each species undergoes during its existence through events in the life of its members, plus the nonphenotypic expression of its genome.' Thus, to study microbial biodiversity, it is necessary to understand interactions between and among species in a given habitat. According to Erwin [20], 'how these species are grouped as a living unit' in a given ecological unit, is a task that is heroically difficult to complete for microorganisms, without a massive, globally coordinated program of action.

Microorganisms are the ubiquitous janitors of the Earth, occurring in all climate areas, including those once considered to be most unlikely to support life—the cold of the Arctic and Antarctic, the heat of geysers and oceanic hot vents, and deep within rocks. They are decomposers, converting nutrients in organic wastes and from dead organisms into molecules that are reused within ecosystems. Many of them live symbiotically with, in, and on higher organisms. Microorganisms also include agents that cause disease, in some cases, maintaining ecological balance, in others, decimating host populations. Gold [29] suggested the following general rule: 'that microbial life exists in all the locations where microbes can survive.' He went on to state that there are no sites on Earth that have been free of microbial 'infection' for long periods of geologic time.

New findings

Our knowledge of extant microbes is ever increasing [8] and the range of microbial diversity has proven to be extraordinary. In the early 1980s, following the discovery of the deep sea hydrothermal vents, Stetter *et al* [47] identified hyperthermophilic prokaryotes from hot springs and volcanic fields, as well as from shallow and abyssal marine hydrothermal vents. Some of these organisms, such as the sulfate-reducing *Archaeoglobus*, have been classified in the domain (classification higher than kingdom) *Archaea* [56]. Furthermore, representatives of the *Archaea* have been found to be widely distributed in the world oceans. Since

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the early identifications, many members of the *Archaea* have been identified and classified [55] and some produce enzymes that have, or are expected to have, commercial utility [52].

The microflora of the Antarctic has been studied intermittently since the turn of the century, but discovery and identification of cold-tolerant species of protists, cyanobacteria, microalgae, bacteria, and yeasts have been significant since the 1980s [23,59]. Appreciation of pyschrophiles, the 'cold-living bacteria,' has extended to activities of these bacteria at temperatures close to freezing. Many contain powerful proteases active at very low temperatures. Furthermore, the discovery of procaryotes that have not yet been cultured, but can be detected in both the Pacific and Atlantic Oceans in large numbers opens a totally unexplored vista.

Also, since the 1980s, knowledge of the deep terrestrial microorganisms has increased exponentially [22]. Bacteria and other microorganisms isolated from deep drilling cores have proven to be new species with unusual physiological properties.

In the marine environment, there is some of the greatest diversity of animal and plant phyla on Earth, but this region is the least known of all ecosystems [10]. Giovannoni *et al* [27], using an analysis of ribosomal RNA clones, detected significant diversity among the bacterial picoplankton from the Sargasso Sea, finding a novel lineage of Sargasso Sea bacteria. The data support the now widely-held view that microbial ecosystems contain novel, as-yet-uncultivated species. Giovannoni *et al* [27] also hypothesized that viral infections could be a contributing factor to selection pressures in these species. In fact, viruses recently have been found to be more common in marine ecosystems than had been known previously [6,25,26,39,57,58]. It is now hypothesized that they play a significant role in the global carbon cycle [11]. An interesting finding in the Chesapeake Bay is that during late summer and early fall, viruses outnumber bacteria [57].

Fuhrman *et al* [25,26] identified several unique microorganism clones in marine samples collected from the western California Current area of the Pacific Ocean and from the Atlantic Ocean off the coast of Bermuda. These appear to represent new bacterial taxa, notable among them are members of the *Archaea*.

Coral reefs harbor abundant microbial species, some of which demonstrate antiviral activity [15,44,45]. In fact, the structure of marine invertebrates, eg, sponges, appears to comprise a structural composition dependent upon procaryotes. Symbioses are also a unique feature of marine bacteria and invertebrate animals. From these invertebrate–bacterial interactions have come some interesting new metabolites of potential pharmaceutical value.

Previously unknown organisms comprising the microflora of vertebrates have recently been identified. *Epulopiscium fishelsoni*, the largest bacterium yet discovered, lives within the intestinal tract of the brown surgeonfish, *Acanthurus nigrofuscus*, found in the Red Sea. Other similar, but not as large, bacteria, are symbionts within surgeonfish from Australia's Great Barrier Reef [1]. *Fibrobacter* are a diverse group of fiber-digesting bacteria found within the gastrointestinal tracts of ruminant and nonruminant herbivores. A recently published study by Lin and Stahl [34] identified a new *Fibrobacter* population within the cecum of a pony.

The bacterial genus *Spiroplasma* occurs as a symbiont only within insects. Whitcomb and Hackett [54] believe that spiroplasmas, which were identified less than 25 years ago, may show the greatest taxonomic diversity of all microbes, another example of unusual microorganisms in new niches.

New genera, species, and strains of fungi are being discovered and some are described in a recently completed book on the systematics of 900 known species within the genus *Phomopsis*, which lives on plant hosts and, in some circumstances, is triggered to cause disease in plants [5]. The fungi have been more extensively described and characterized than the bacteria, with fungal species outnumbering bacterial species more than 10-fold. Thus, investigators studying microorganisms appear to be in a race to discover more species before the environments of the microorganisms under study are forever altered or lost.

Microbes are necessary for life as we know it

Few individuals outside the community of microbiologists discuss the conservation of species that cannot be seen without microscopes. Yet, microbiologists fear the possibility of loss of these organisms whose structure and function may never be studied before they become extinct. Because it is already known how important many microorganisms are to the health of ecosystems of the Earth, it becomes a race with time!

Microorganisms are the 'janitors of the Earth,' degrading dead organisms and recycling nutrients, making them available for other organisms to live and grow on. They destroy wastes and pollutants, again recycling valuable nutrients and releasing them back into the ecosystem, or up the food chain. Hawksworth and Mount [31] write, 'Biodiversity is consequently essential, maintaining the stability of living systems around us, and ensuring that we survive the death, dung, and detritus of contemporaneous members of our own species as well as of other organisms.'

For example, these detritus-consuming microbes aid in recycling of nutrients from between 67% to 88% of the plant material produced in deserts. Although they are aided by arthropods, nematodes, and some rodents, the microorganisms are irreplaceable in the food web.

Microorganisms live symbiotically within the guts of higher organisms, aiding in the digestive process and in fermentation of wastes [35,50]. They dwell on human and animal bodies and within orifices of these bodies. Most of the normal flora are nonpathogenic or become pathogenic only under specific conditions. Goatcher *et al* [28] studied the intestinal microflora of black bears (*Ursus americanus*), grizzly bears (*U. arctos*), and the plants within their environment, isolating 30 genera of bacteria and 16 genera of fungi from grizzly bears, 28 bacterial genera and 13 fungal genera from black bears, and 17 bacterial genera and 16 fungal genera from plants.

Fungi and other microbes live commensally in plant root systems, aiding in cycling of nutrients [31]. Some fungi comprise the bottom of the food chain and are eaten by insects that, in turn, are eaten by higher organisms [31]. Fungi and bacteria also may function as biological pest control agents, preventing harmful bacteria, insects, and nematodes from destroying plants [31]. Some species of naturally occurring bacteria in rice fields, for example, inhibit the growth of *Fusarium moniliforme*, the etiologic agent of bakanae disease in rice [42].

These relatively new findings indicate the diversity and essential nature of microorganisms to the biosphere. Naturally-occurring microorganisms can be utilized effectively in a variety of processes and genetically-engineered microorganisms can produce useful, even essential products. Bread, wine, cheese, bean curd, soy sauce, beer, and other alcoholic beverages are all products of microbial fermentation. Without antibiotics derived from fungi and bacteria, most infections would be fatal. And, without industrial enzymes, many derived from microorganisms, our clothes would not be as clean, our food supply would not be as diverse, and we would not have ready access to inexpensive, effective vitamins and pharmaceuticals. We would have no sophisticated diagnostic kits to test for disease, our environment could not be cleansed, and even wars may not have been won for lack of munitions. Research supplies of RNAses, DNAses, polymerases, and other enzymes have even changed the legal system.

Techniques for studying microbial biodiversity

Once it is determined that an effort must be made to study microbial diversity, the next question is what is the best way to accomplish this objective? Ehrlich and Wilson [18] referred to bacteria as ‘a terra vitae incognita because of the astonishingly small amount of research devoted to their diversity, as opposed to the genetics and molecular biology of select species.’ Clearly, this must be changed, for all microorganisms, not just the procaryotes.

New techniques allow for environmental screening to determine the presence of nucleic acids within environmental samples. These molecular genetic techniques allow screening of organisms that could be maintained in culture along with those that cannot be identified by standard means because they cannot be cultured. Pace [4], for example, extracted DNA directly from samples, then used the polymerase chain reaction (PCR) to amplify small subunit ribosomal RNA (rRNA) genes, selectively amplifying those found in archaea and eucarya. He and his colleagues then compared the ribosomal DNA sequences with known rRNA sequences. Although not allowing the description of specific organisms, this technique permits determining numbers and lineages of microorganisms within environmental samples, notably phylogenetic relationships and genetic similarity to sequences in established databases.

Although workers in many laboratories amplify 16S rRNA genes from environmental samples, and have produced important results from such work [25,26,27], even with simplification of steps, this is still a time-consuming technique not suitable for analysis of hundreds of samples. It also requires a relatively large database [14].

It is now possible to study microbial communities, such as those in the Chesapeake Bay, using the randomly-amplified polymorphic DNA (RAPD) method [40]. This generates DNA fingerprints characteristic of the community in question. Like the rRNA gene method, this does not allow for individual identification of organisms. It does, however, allow screening and comparisons of organisms in different sample areas over time.

This kind of community screening, if carried out in the same area on a regular basis, may allow us to assess changes in microbial biodiversity at a particular site or in a particular ecosystem. This could constitute an early warning system for environmental changes [12].

Others have utilized DNA reassociation rates, in which denatured single-stranded DNA (ssDNA) is reassociated under conditions with controlled temperature and monovalent cation concentration. Reassociation rates give an indication of the heterogeneity of the sample [51].

More specific probes and gene sequencing, along with more traditional taxonomic methods, such as phenotypic characterization, microscopy (with or without monoclonal antibody tags), plating, and culturing, may be necessary to identify microbial species.

One major question, however, is: Should we look solely at taxonomic diversity, or would it be more useful to study function diversity? Zak *et al* [60] suggested that functional diversity would be a better way to study the roles of microorganisms in the environment than looking at taxonomic diversity. They adapted the Biolog microplate identification system, which allows for detection of specific Gram-negative or Gram-positive bacteria on microplates, allowing study of functional diversity of bacteria in soil samples from plant community types. They did, however, point out that this technique does not substitute for DNA or 16S rRNA methods, because the Biolog system only identifies organisms that can grow on the media. Furthermore, the Biolog system has deficiencies in identifying fresh environmental isolates. The question of the as-yet-unculturable microorganisms also must be considered. The study of functional diversity using such methods is limited, but development of techniques to allow both screening and identification for a fully complete biotic survey will be necessary.

Viruses in marine samples can be studied by nonmolecular methods: transmission electron microscopy (TEM) and epifluorescent microscopy (DAPI stain) with video [25,26] and by differential filtration [58]. The development of improved methods for isolating and characterizing viruses in the marine environment now makes it possible to determine the role of viruses in microbial communities.

Conservation initiatives

Does it matter if microbial diversity is lost to incursions of humans on the environment? The answer is clearly affirmative.

Hiding within the as-yet-undiscovered microorganisms are cures for diseases, means to clean polluted environments, new food sources, and better ways to manufacture products used daily in modern society.

International guidelines and agreements include protection for microorganisms. *Caring for the Earth* [32], an update of the World Conservation Strategy presented in 1980, presents a blueprint for preserving the environment and biological diversity. Although it does not specifically address the issue of preserving microbial biodiversity, it encourages establishment of protected areas that would protect all organisms within them, and encourages energy production from biomass, use of biological pest control, maintenance of good soil conditions for agriculture, and support for taxonomic and systematics research. All of these objectives, if achieved, will have a positive impact on the study and maintenance of microbial biodiversity.

The Convention on Biodiversity, signed in Rio de Janeiro, Brazil, in 1992, and effective December 1993, covers microbial biodiversity and includes the Microbial Diversity 21 initiative [30]. The General Agreement on Tariffs and Trade (GATT), concluded by the Uruguay Round of Negotiations in 1994, specifically requires signatory nations to adopt a system of patenting for microorganisms [16]. The Trade-Related Intellectual Property section (TRIPS) of GATT states that there must be intellectual property protection both of (genetically engineered) microorganisms and microbiological processes.

At the national level, initiatives may be carried out to promote the conservation of microbial biodiversity. The United Kingdom, for example, is interested in decreasing the amount of land being used for intensive agriculture, setting it aside for future use. This is expected to increase knowledge of the microbial biodiversity of this land area [48]. The US National Science Foundation (NSF) proposed a national research initiative on Biological Diversity in Marine Systems (BioMar) that would stimulate research in systematics of marine species, including microorganisms, and ecosystem studies [9].

Hawksworth and Mound [31] pointed out that completing a worldwide inventory of biota requires additional taxonomic collections, training of people in many different countries to carry out taxonomic studies, and serious international coordination of these efforts. The African Network of Microbiological Resources Centres (MIRCENs) is a step in that direction [17]. A new MIRCEN, focusing on microorganisms used in biohydrometallurgical processes has been established in Pune, India [2].

Not only are culture collections important, but in a world united by electronic communication, computer databases may supply invaluable information to taxonomists and other researchers. A move to link several microbiological databases and add functional data to the genetic data, was explored at the recent International Symposium on Microbial Ecology in Santos, Brazil [53].

What is the risk if action is not taken?

There is concern within the community of microbiologists that, due to funding and other constraints, projects having immediate utility have taken priority rather than the hard work of taxonomy and microbial ecology [7]. If research is continued in this mode, the opportunity to begin the serious task of determining the presence and function of microorganisms in the environment will be lost. At present, there are only *ca* 5000 nature reserves or other protected areas, comprising only 3% of the Earth's land [46]. The trend in the US is to decrease protected lands, and there is reason to assume that less developed nations, citing their own financial problems, will follow on the path of the US.

Major ecological changes already are occurring that affect the health of humans and animals worldwide. Baquero [3] stated, ‘A microbiologically damaged environment probably represents the most relevant pathogenic factor and consequently one of the biggest challenges in public health sciences.’ Friend [24] wrote, ‘Disease is often an expression of out-of-balance systems.’

Advantages to studying microbial biodiversity

In order to best exploit microorganisms, we need to know what is there and what we can use [8]. Studies of related organisms may yield potential products. For example, species of the actinomycete *Kitasatosporia* have been found that produce a herbicide, and an antibiotic (setomimycin) [8]. Microbial and fungal secondary metabolites may be sources of new chemical and pharmaceutical products [38]. Antibiotics have been the major products from microbial screening, with some 10 000 discovered in the last 50 years [36]. More recently, microbes are being probed for compounds with antifungal, antiviral, or antitumor activity. Other drugs, like mevinolin, which reduces cholesterol in humans, have been found through microbial screening [36]. Fungi are a potentially rich source of active molecules. Some fungi produce toxins that protect the plants on which they live from predation [36]. Other fungi produce insect toxins that are potential biological control agents.

Organisms have been found that can accumulate the heavy metal gallium [8]. Microbes, especially chemolithotrophic bacteria, like *Thiobacillus ferroxidans*, and *T. thiooxidans*, are increasingly used in mining for controlled bioleaching of metals [41]. Uses for microbes and microbial products appear to be nearly endless. But, if many microbial species are lost, there is one intangible benefit that would be lost forever: the knowledge of who we are and where we came from. The remarkable discovery of the archaea, and subsequent work with the newly discovered members of this taxon, are yielding clues to the origins of life on Earth [52]. Pace (personal communication) currently is describing a new kingdom within the archaea, the Korarchaeota (translated as ‘the very youth of life’). Members of this kingdom may be the phylogenetic bridge between the eucarya and the phylogenetically deep-rooted archaea. Without such scanning of environmental samples for microorganisms, this ‘bridge group’ would remain unknown.

Unless we find ways to continue screening and studying microorganisms and preserving their habitats, there is no way to know the functions of these organisms within their natural habitats, and no way in which to understand the evolved richness of life. A major task is to educate the public, whose knowledge of microorganisms may be limited to knowing that microorganisms can cause disease. The public must understand that, although not ‘cuddly’ like the panda, or as impressive as the California condor or African elephant, microorganisms are essential for the continued existence of human populations on Earth.

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