This report is based on an American Academy of Microbiology colloquium held January 19-21, 1996, in Palm Coast, Florida. The colloquium was supported by the National Science Foundation, the National Oceanic and Atmospheric Administration of the U.S. Department of Commerce, the U.S. Department of Energy, and the American Society for Microbiology.

**Cover photo:** These bacteria were collected from the lower depths of a lake that was depleted in oxygen. Most of the organisms shown here are anaerobic purple-sulfur or green-sulfur photosynthetic bacteria that differ from algae and plants in that they do not evolve oxygen during photosynthesis. These anoxygenic photosynthetic organisms are believed to be descended from some of the first photosynthetic bacteria of Earth's biosphere. They must be high enough in the water column to obtain light, yet low enough to obtain hydrogen sulfide needed by them for photosynthesis.

*(Courtesy of James T. Staley, University of Washington)*
The Microbial World: FOUNDATION OF THE BIOSPHERE

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Preface

Explosive developments in microbial ecology have occurred in recent years, notably at the molecular level. Newly discovered habitats are being explored, exemplified by the deep sea hydrothermal vents, that are yielding strange and unusual microorganisms. Evidence for life on Mars has been presented by scientists studying meteorites, with the life forms suggested to be microorganisms. Clues to human evolution also are being detected in the ancient microbial life studied by a variety of new methods, but most dramatically by nucleic acid sequencing. The cascade of information concerning microbial diversity offers a richness for biotechnology applications. Sadly, the human resources, as well as the instrumentation and laboratory facilities for microbial diversity research on exploration and harvesting of microbial diversity for biotechnology applications, are seriously lacking.

A colloquium addressing this rapidly expanding area of microbiology, namely molecular microbial ecology, focused on microbial diversity discovery and harvesting, was convened by the American Academy of Microbiology at Palm Coast, Florida, January 19-21, 1996. A call for action is contained in the series of recommendations provided in this report. The information, gathered by the team of experts who deliberated the issues, has been succinctly presented. Opportunities will clearly be lost if the exhortations of the colloquium go unheeded.

Gratitude is expressed to all who participated in the colloquium. Each participant brought valuable advice and expertise and all worked collectively to produce the report. The Steering Committee deserves a special note of thanks for their hard work in distilling the information and discussions into a strong message to the scientific community, national agencies whose interest in the topic is important to their missions, and the general public. Finally, special acknowledgment is made to Dr. James Staley, Chair of the Colloquium Steering Committee and ardent proponent of microbial diversity.

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Executive Summary

A revolution has occurred in microbiology, the ramifications of which are not yet widely known or appreciated, but which provides new opportunities for agriculture, medicine, and industry. Using new techniques and approaches—in particular sequencing and analysis of ribosomal RNA genes—microbiologists have discovered that there are at least 20 major evolutionary groups of the microbial life forms on Earth—bacteria, fungi, algae, and protozoa—that are even more diverse than the better known plant and animal kingdoms. Furthermore, recent evidence indicates that millions of microorganisms still remain to be discovered. These new microorganisms provide a vast untapped reservoir of genetic and metabolic diversity, the harvesting and study of which will have far-reaching, positive effects for society in areas such as enhanced food production, medicine (e.g., antibiotic discovery), bioremediation of waste materials, and agriculture.

Part of the revolution in microbiology stems from development of methods that now make it possible to inventory the diversity of microbial life on Earth and to do so cost-effectively. The power of the new technologies on which these methods are based will reveal the extraordinary range of metabolic, physiological, and evolutionary diversity of microbial life on Earth. Programs to harvest this bountiful, yet invisible, microbial diversity have already begun in Japan and Europe. The United States scientific community, largely responsible for creating the revolution, is uniquely positioned to mount a comprehensive effort that takes full advantage of newly evolving technologies. A microbial exploration and harvesting program to assess the variety and commercial potential of microbial life forms on Earth will reap great benefits for society. Furthermore, a microbial exploration and harvesting program employing existing and developing technology would launch a voyage of discovery and exploration that would quantify the diversity of microbial life forms on Earth and provide, at least in part, an answer to the question of how much microbial diversity has yet to be discovered. Current estimates, based on partial and random sampling, are certainly gross underestimates of the actual diversity of microbial life extant on Earth. An inventory would identify environments where the greatest diversity exists, and those areas can be productively targeted for more intensive exploration. Harvesting and study of novel microbial types will doubtless lead to the discovery of new biomaterials, development of new commercial processes and enterprises, and acquisition of knowledge of practical importance to society.

To grasp the full extent of microbial diversity, iterative steps need to be taken. For example, sequencing the genome of at least one microbial species from each of the major phyla of microbial life can provide information about another type of diversity: the biochemical potential of microorganisms comprising each of the major groups. DNA sequence analyses of microbial genomes obtained to date have revealed an unanticipated richness of novel genes; this approach can be expected to identify completely novel processes and biochemical compounds that will find timely application in biotechnology and health care.

In order to delineate effectively the extent of microbial diversity and identify potential applications, several recommendations are made, addressing infrastructure necessary to ensure attainment of the goals, and include initiatives in education and training, instrumentation needs, establishment of regional and national research centers of excellence, development of databases, and enhancement of genetic resources in culture collections.
Introduction

Although most microorganisms are too small to be seen, their importance cannot be ignored. Microorganisms are the foundation of the biosphere—both from an evolutionary and an ecological perspective. Microorganisms were the first organisms on Earth; they have lived on this planet for a period of at least 3.7 billion years of the 4.6 billion-year existence of the Earth. Microorganisms were living inhabitants for more than 3.0 billion years before the appearance of plants and animals. Not only did plants and animals evolve rather recently in Earth's history, but they evolved from microbial ancestors. A recent report of evidence for microbial life on Mars also is consistent with the concept that microorganisms preceded plants and animals on Earth.

The Earth's biosphere is largely shaped by geochemical activities of microorganisms that have provided conditions both for the evolution of plants and animals and for the continuation of all life on Earth. Microorganisms carry out unique geochemical processes critical to the operation of the biosphere. Therefore, it is not surprising that the diversity of microorganisms—from genetic, metabolic, and physiological aspects—is far greater than that found in plants and animals.

In contrast to plants and animals, the diversity of the microbial world is largely unknown (see Table 1), and, of that which is known, the diversity is spectacular. Some microorganisms live at boiling temperatures, or higher, in hot springs and deep sea thermal vents; others live at temperatures below freezing in sea ice. Some produce sulfuric and nitric acids. Many grow without oxygen; the anaerobic activities of these microorganisms are necessary for carrying out the many essential processes in the environment that cannot be accomplished by plants and animals, including methane production and nitrogen fixation. Such familiar activities as leavening bread and production of yogurt, pickles, wine, beer, and cheeses rely on microorganisms carrying out the key processes.

Microorganisms also play other essential and beneficial functions for society. For example, we rely on them for production of antibiotics, antitumor agents, and a variety of biotechnology products (see Table 2). We use microorganisms to produce human insulin via genetic engineering and to provide enzymes for manufacturing. They are important in agriculture; their metabolic activities enhance soil fertility, especially in their often unique roles in the nitrogen, phosphorus, sulfur, and carbon cycles.

A new awareness of microbial diversity has developed in recent years. Advances in molecular biology have allowed biologists to compare all living organisms to one another on the basis of highly conserved genes. Initial studies focused on those genes that code for ribonucleic acid (RNA) of the ribosome, the cellular structure responsible for protein synthesis in all organisms. In particular, the sequence of the bases of the small subunit (16S or 18S) of ribosomal RNA (rRNA) has been used to map the relationship of all living organisms (see Figure 1). The phylogenetic tree shows the extraordinary diversity of microorganisms. Figure 1 also

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1 The colloquium held to develop this report was dedicated to Carl Woese for the role he has played in developing our understanding of the evolutionary tree of living organisms.
illustrates that, like the plant and animal kingdoms, microbial groups also show equally deep branching, that is, an ancient evolutionary separation. Thus, there are approximately 12 phyla of Eubacteria (or true bacteria), three phyla of Archaea (previously called archaebacteria), and several phyla of other microorganisms (fungi and protists).

It is now also known that many more forms of microbial life exist on Earth than previously expected. Indeed, most of the organisms from natural soil and aquatic communities have not yet been grown in culture and characterized. Thus, in contrast to plants and vertebrate animals in which 85 to 90% have been described, it is conservatively estimated that less than 1% of the bacterial species and less than 5% of fungal species are currently known (see Table 1). The universal tree of life does not as yet include the latter. However, newly developed molecular techniques can be used to identify those microorganisms from the environment that cannot be cultivated. Furthermore, recent advances in the cultivation of microorganisms indicate that many of these organisms can be grown and compared to known microorganisms. Microbiologists can now use these new tools and procedures to explore and quantify the extent and variety of previously unrecognized life forms that exist on the planet, the last great frontier for biology on Earth.
Importance of Microorganisms to the Biosphere

Microorganisms are the foundation of the biosphere. Without them, other life forms would not have evolved and could not exist. Microorganisms established the geochemical conditions on Earth that enabled evolution of plants and animals. Plants and animals are descended from microorganisms, and their cells are now known to be composites of microorganisms. For example, the mitochondria of all plants and animals are derived from bacteria. Similarly, the photosynthetic organelle, the chloroplast, found in all plants and algae is descended from a group of photosynthetic bacteria, the cyanobacteria. Cyanobacteria are believed to be the first organisms on Earth to produce free oxygen gas and, concomitantly, the protective ozone layer around Earth, thereby providing conditions for evolution of land plants and animals.

Humans and other animals, as well as plants, are completely dependent on microorganisms for life. Like all animals, humans harbor billions of microorganisms in their digestive tracts, microorganisms necessary to digest food and provide nutrients, such as vitamins and amino acids, for growth and a source of energy. Plants also require microorganisms to provide nutrients for growth, an activity that takes place largely in root systems. There the organic materials in soil are broken down by bacteria and fungi to provide inorganic materials, such as nitrogen and phosphorus, the natural fertilizers made available by microorganisms and required by plants for growth and development.

Microorganisms exist everywhere physical conditions permit. Although lake water may appear transparent to the eye, a liter of the water can harbor a billion bacteria. A gram of soil can also contain over a billion bacteria. Many microorganisms have special dispersal cells that can be carried by winds across and between continents. In addition, birds and insects transport microorganisms as they fly. Thus, we live in a world teeming with microbial life that carries out a myriad of activities essential for sustaining the biosphere of Earth.

Microorganisms are highly diverse genetically and metabolically, far more so than plants and animals. This should not seem surprising because microorganisms have existed on Earth for over 3.5 billion years, whereas multicellular plants and animals have existed only 600 million years. From analyses of molecular sequences of genes, such as 16S and 18S ribosomal RNA, approximately 20 separate, main phylogenetic groups of microbial life have been identified, comparable in depth and breadth to the animal and plant kingdoms (Figure 1; Woese 1994; Sogin 1994; Sogin et al. 1996a, 1996b; see also Table 5). Furthermore, microbiologists have discovered groups that represent new phyla, such as the Korarchaeota, not yet studied in pure culture (Barnes et al. 1996).

One of the most surprising characteristics of microorganisms is the range of physiological conditions under which they flourish. They grow across broad ranges of temperature, pH, salt concentration, and oxygen concentration (see Table 3). Some thrive at boiling temperatures in hot springs and at temperatures higher than 100ºC in submarine vents. Others are found in sea ice off Antarctica and at the North Pole. Some produce sulfuric and nitric acids, and many microbial species live without oxygen. Others live in saturated salt brines, and some are resistant to high levels of radioactivity.

The variety of metabolic types of microorganisms is enormous. Some are photosynthetic and, like plants, produce oxygen in this process. In fact, this “biotechnology” first occurred in the cyanobacteria, which subsequently evolved endosymbiotically to form chloroplasts that enable algae and plants to conduct photosynthesis. Other bacterial groups carry out photosynthesis by different pathways and produce products such as sulfur. Microorganisms are the primary, if not sole, agents responsible for degradation of a great variety of organic compounds, including cellulose, hemi-
cellulose, lignin, and chitin (the most abundant organic matter on Earth). If it were not for microbial activities involved in natural decay, excessive amounts of organic matter would accumulate in forests and aquatic sediments. In addition, microorganisms are responsible for degradation of toxic chemicals derived from anthropogenic sources, such as PCBs (polychlorinated biphenyls), dioxins and other pesticides. Because microorganisms are so versatile, they are relied upon to digest wastes in sewage treatment plants, landfills, and toxic waste sites. It is in this regard that the field of bioremediation, encompassing all of these processes, is still in its infancy. Much needs to be learned before microbial breakdown processes can be controlled and enhanced in situ.

Microorganisms play important roles in geochemical processes. For example, the global nitrogen cycle in nature is dependent on microorganisms. Unique processes carried out by microorganisms include nitrogen fixation (the natural conversion of atmospheric dinitrogen gas to utilisable organic cell nitrogen), oxidation of ammonia and nitrite to nitrate, and nitrate reduction with formation of dinitrogen and nitrous oxide gases. Similar important and unique roles are played in other cycles, such as the sulfur and carbon cycles, as well as in the oxidation and reduction of metals. If it were not for microorganisms, substances such as cellulose and lignin would not be recycled; they would accumulate in the environment. Indeed, almost all organic substances are recycled via activities of bacteria, fungi, and protozoa.

The importance of microorganisms in agriculture is enormous and extends beyond geochemical cycles. Indeed, most of the fertility of soil is derived from microbial mineralization and in production of nitrogen for plant growth. These processes extend to lichen- and cyanobacterial-dominated soils which occupy a larger surface area on Earth than in tropical rain forests. Mycorrhizal fungi form important rhizosphere associations with almost all plants. Such associations are essential for optimum growth and, in fact, permit some plants to grow in areas they could not otherwise colonize. Recent advances in agriculture stem from breakthroughs in the genetic engineering of plants; one of the most dramatic examples is that of the bacterium Agrobacterium tumefaciens. Normally the causative of crown gall disease in plants, this bacterium has been used to transfer favorable properties into an agriculturally important plant species, thereby providing a mechanism for introducing genes that provide resistance to plant diseases, insects or pesticides into plants. Microorganisms are important in recycling waste materials. Sewage (wastewater) treatment and the breakdown of garbage in landfills occur because of microorganisms. These microorganisms do this “for free” because, in most cases, they derive energy from the process.

A recent discovery indicates that microorganisms may influence weather. Some marine algae produce dimethyl sulfide (DMS). This compound is volatile and escapes into the atmosphere where it is photo-oxidized to form sulfate. The sulfate acts as a water nucleating agent and when enough sulfate is formed, clouds are produced; these clouds have three major impacts. First, they shade the ocean and, thereby, slow further algal growth and DMS production, eventually decreasing cloud formation. Second, the clouds lead to increased rainfall. And third, because clouds are reflective of incoming sunlight, the clouds reduce the amount of heat that reaches Earth, moderating global warming.

Microorganisms are at the core of biotechnology. Many antibiotics and anti-tumor agents are derived from microorganisms, including penicillin, streptomycin, and chloramphenicol. The emergence of multiple antibiotic-resistant pathogenic bacteria has necessitated the search for new antibiotics. Because there are so many types of microorganisms, they produce many unique products currently useful in biotechnology and offer great promise for exploitation in the future.

Microorganisms are the foundation of the biosphere. Without them, other life forms would not have evolved and could not exist.
3.1 Exploration and quantification of the extent and variety of microbial life forms on Earth

During the more than 3.7 billion years that life has existed on Earth, evolution has led to extensive diversification in microorganisms, and, therefore, it is not surprising that microorganisms exhibit the greatest metabolic diversity. To date, scientists have explored only a small fraction of microbial species diversity, and most of the microbial world remains undiscovered.

Microbiologists have only just begun to discover the variety and abundance of microbial life on Earth. What is already known comprises compelling evidence that microbial life is extremely diverse—genetically, metabolically, and ecologically. Now that microbiologists can classify microorganisms into a meaningful phylogenetic system, it is possible to explore microbial diversity within natural communities. This diversity is much greater than that of large organisms (Figure 1), even though the number of described species of macroorganisms, particularly insects, greatly surpasses that of microorganisms (Table 1). This situation is attributable, in part, to the lack of emphasis placed on research in microbial taxonomy and the nature of the analyses used to describe microbial species before the new methods of molecular biology were available.

There are many aspects of microbial associations that have just begun to be explored. Microbial symbioses with animals, plants, and other microorganisms are diverse and common in natural communities. These important symbioses are essential to the livelihood of both the microorganism and its partner. Without microbial symbionts, most animals and plants could not survive in natural communities. Thus, studies of all types of microbial symbioses are important to determine the nature of the chemical and functional interdependencies between symbiont partners.

There is an awareness that much remains to be learned about microorganisms found in soil and marine environments, but few people appreciate how little is known about another type of environment, the intestinal tracts of invertebrates, lower vertebrates, and mammals. The intestinal tract of mammals contains one of the most densely structured communities found anywhere in nature. The microbiota of the human colon is fairly well characterized, although some of the dominant species have still not been cultivated (Salyers 1986). Much less is known about the microbiota of the female vaginal tract, despite the importance of the microbiota as a barrier against infection. The only other animal in which the microbiota has been well studied is the cow. The bovine rumen, which is agriculturally important because it allows cows to convert otherwise indigestible grass or silage into usable energy (Hungate 1966) has been extensively studied. However, less is known about the microbiota of other farm animals. The investment made in characterizing the microbiota of humans and cattle has resulted in major advances in human and animal health and nutrition.

The discoveries that most ulcers are caused by bacteria and that periodontal disease is of bacterial origin raise the possibility that both of these diseases can be cured by antibiotics. Studies of human and animal microbiota have also spawned a multimillion dollar industry, probiotics. Probiotics are preparations of live bacteria that are ingested or

Microbiologists have only just begun to discover the variety and abundance of microbial life on Earth... microbial life is extremely diverse—genetically, metabolically, and ecologically.
applied to bolster or supplement the effectiveness of the natural microbiota as a protective shield against infection. A better understanding of microbial symbiont diversity will lead to a more rational development of probiotics and, thereby, to another, less intrusive, mechanism for controlling and curing disease.

Relatively little is known about the microbiota of invertebrates and lower vertebrates. Some, such as the squid and luminescent fishes, have unique organs that have evolved to contain specialized populations of bacteria which provide a light source for these marine animals. Owing to their abundance and dominant role as tropical herbivores, investigations of the intestinal microbiota of insects, such as termites responsible for cellulose digestion, are underway in several laboratories. The types of microorganisms residing in the intestinal tracts of most animals is largely unexplored and deserving of much greater attention, inasmuch as these animals are completely dependent on their microbial symbionts to carry out anaerobic digestive processes that provide them with food (Kane and Pierce 1994). A recent example is the discovery of Archaea in the gut of Holothurians (sea cucumbers) from abyssal marine samples; McInerney et al. (1995) found novel, as yet uncultivated, deeply branching Archaea that may be normal inhabitants of the mid-gut of these animals. Another is the recent discovery of the largest known bacterium in the intestines of the reef surgeonfish (Angert et al. 1993). Studies of intestinal microorganisms should be coordinated with systematic studies of their hosts to determine the degree to which microorganisms and their hosts are cospeciating along shared evolutionary trajectories.

Human and animal symbiont research should be coordinated with other census studies. An initial inventory might well proceed along the same lines, using in situ PCR for the whole community and cultivation (Wilson and Blitchington 1996). Key species of vertebrate and invertebrate animals should be selected for study in consultation with experts on the macroecology of the site. An effective process for analyzing the microflora of animals should include sections examined by microscopy and in situ fluorescence hybridization to determine whether there are extraintestinal sites colonized by microorganisms. Once a baseline for the microbiota is established for a particular type of animal, that animal can be monitored over time to determine changes due to seasonality, diet, and age (Salyers 1995).

In addition to the above, there are other microbial associations with other animals and plants that also await more thorough investigation. These include fungal mycorrhizal associations, algal and bacterial associations with marine animals, and consortial associations among microorganisms involved in bioremediation. A notably striking example comprises the so-called “hot-spots” on Earth. Hot-spots are areas where endemic plants live. According to a recent report (Myers 1990), 20% of all plant species, i.e., plant diversity, is confined to 0.5% of the Earth’s land surface. Many of these hot spots are threatened with imminent destruction and loss from human intrusions. Moreover, only limited knowledge of and access to most of the areas where these endemic species live is available (Pimm et al. 1995). Since each species of plant is expected to harbor a variety of symbiotic species of fungi and other microorganisms, destruction of an endemic plant species in a hot-spot will result in loss of associated microbial species as well.

Study of symbiotic microorganisms will provide an understanding of which species of microorganisms are important symbionts of humans and other animals, plants, and microorganisms. The question
whether humans have unique microbial species as symbionts needs to be addressed, as well as examination of roles these symbionts might be playing in human health. As mentioned above, bacteria and fungi live on or in other animals, such as insects, and are important for their livelihood. Microorganisms have developed such symbioses with plants also. Indeed, it is estimated that 85% of all plants have mycorrhizal fungi. Furthermore, fungi live in close association with mosses and are equal partners in lichen symbiosis with algae. Fungal/fungal symbioses also occur. Bacteria, such as *Rhizobium* and *Frankia* spp., live in nitrogen-fixing associations with plants—symbioses that are still poorly understood. Bacteria, too, often live in close association with other bacteria, particularly in anaerobic environments where synergistic metabolic interactions among microbial groups may be very tightly coupled.

With the procedures that have recently been developed for cultivation and identification of bacteria and other microorganisms from environmental samples, microbiologists are able to assess the full diversity of microbial life forms on Earth. Several approaches can be taken to achieve such a goal, and these are described individually below.

### 3.1.1 A Molecular Microbial Inventory (MMI)

Access to, and, therefore, the ability to study, natural microbial ecosystems has been limited by a traditional, fundamental obstacle: the inability to cultivate most naturally occurring microorganisms using standard techniques. It is estimated that cultivation procedures yield less than 0.1% of the total bacteria from typical natural environments (Staley and Konopka 1985). Most of the remaining 99.9% appear to be metabolically active as determined by microautoradiography and tetrazolium dye reduction assays (Tabor and Neihof 1984). Therefore, it is no longer possible (or useful) to rely exclusively on viable cultivation to assess microbial diversity in natural habitats.

In light of these limitations, how should microbial diversity be assessed? Phylogenetic analysis provides the most orderly framework to date for biological classification. Over the past several years, reasonably facile procedures have become available for identifying microorganisms by techniques of molecular phylogeny. Differences in the sequences of equivalent genes from different organisms are used to infer evolutionary relationships. An assessment of the phylogenetic type (phylotype) of an organism provides considerable perspective as to its nature. Some properties of an organism can be predicted, based on properties of its cultivated relatives. Since phylogenetic assessment is based on sequences, it does not depend on isolation of the microorganism from an environment. Thus, native microbiota can be identified tentatively and characterized by sequencing the rRNA genes in DNA extracted directly from the environment.

Armed with such new and powerful techniques, microbiologists can determine which species are most abundant in natural communities. A two-pronged complementary approach is advised in investigations of the abundance and variety of microorganisms in natural habitats.

A first approach could employ new techniques to identify microorganisms from natural communities without cultivating them. This breakthrough procedure circumvents the difficulty microbiologists have had in cultivating most microorganisms from natural environments. This will result in the development of a library of 16S rDNA types from a variety of natural communities to determine the types of organisms that are present. Furthermore, this approach can also be used to identify newly emerging pathogens as well as those which cannot be cultivated from humans, animals or plants.

A second approach could employ recently developed cultivation procedures to isolate representatives of both abundant and rare species for characterization of phenotypic and genotypic features. Strains thereby obtained can provide source material for commercial applications. All isolates representing new species that are characterized by 16S rDNA sequence analysis can be placed in the universal tree of life, named, and classified by specialists. If both noncultivation and cultivation approaches are successful, results obtained should show considerable agreement—that is, the same 16S rDNA sequences representing the predominant organisms from an environment will be...
discovered using both approaches. In situ probing can be used to confirm that the cultivated organisms are, indeed, among the predominant organisms in the environment.

The initial habitats selected for study should be economically or environmentally important, e.g., economically critical agricultural soils. Examples of environmentally important habitats include oceans and their sediments, deserts (because of increased desertification due to global warming), and the little understood habitats, such as hot springs, deep sea thermal vents, and sea ice microbial communities. Additional habitats should be included in microbial discovery and harvest. Specific habitats selected for investigation should be chosen in collaboration with botanists and zoologists so that all taxa, from microorganisms to plants and animals, can be inventoried in selected natural communities.

In a microbial exploration and harvesting program, both non-cultivation and cultivation approaches should be undertaken, as discussed below.

3.1.1.1. Non-Cultivation Approaches

One of the most recent and exciting breakthroughs in the characterization of microorganisms from natural communities comes from procedures used to analyze nucleic acids of bacteria present in the community (Pace 1996). In this approach, which does not require cultivation of the organism, nucleic acids are extracted from the environment, and their ribosomal RNA or rDNA (genes coding for rRNA) are obtained and sequenced to identify the organism. In the simplest variation of this approach, PCR (polymerase chain reaction) is used to directly amplify 16S rDNA from the environment using rDNA primers for the taxonomic group of interest. The PCR products are cloned and the sequence determined and analyzed. The result is determination of the common phylotypes (phylogenetically identifiable taxa) that occur in the environment.

This approach has been successful in identifying novel phylotypes that extend our view of the diversity of microbial life. For example, recent reports have identified phylotypes related to the "hyperthermophilic" and other Archaea from cold and temperate marine and terrestrial habitats (DeLong 1992; Furhman 1992; Pace 1996). Thus, the approach of detecting organisms through their genes is proving very useful for exploration of new life forms.

A concerted effort should be undertaken to conduct a molecularly based census of the types of microorganisms that occur in representative environments—an environmental genotype inventory that would provide the information for identifying predominant phylotypes in communities. A reasonably comprehensive survey of abundant representatives of Earth's microbiota can be achieved with modest effort using automated technology from current whole-genome projects. As an example of scope, it is estimated that the analysis of 1000 clones (to detect the most abundant genome types) from 100 environments would comprise a more modest effort than to sequence the complete genome of a single bacterium, such as *Escherichia coli*.

To manage an environmental genome inventory effectively, accessibility to methodology must be considered. Analysis can be divided into three major phases: (I) DNA extraction, (II) DNA sequencing, and (III) sequence analysis. Phase I can be carried out by many researchers around the world, but special workshops will be needed to increase skills and ensure that common approaches are used. Phase II requires both expensive equipment and specialized staff and cannot be afforded at many sites. Therefore, there must be centralized service facilities in a region for the research (see Infrastructure Needs, Regional and National Centers for Excellence). Phase III will require specialized training in phylogenetic analyses of sequences. Sequences obtained, as well as other information on the location and properties of the collection site, must be placed in a database.

3.1.1.2. Cultivation Approaches

Cultivation of microorganisms greatly enhances the ability of the microbiologist to determine the full complement of genetic and physiological characteristics of newly isolated species. Strains available in pure or mixed culture can be examined to determine what novel features they have, including those that might be of special interest to society. Thus,
this aspect of a microbial inventory is particularly important.

Cultivation is the isolation and multiplication of organisms after removal from the environment. As mentioned previously, it is difficult to recover, in viable, actively growing and reproducing cultures, most organisms from typical soil and aquatic habitats. The explanation of this phenomenon is still largely unknown. However, it may be a result of natural dormancy which would be expected in certain microhabitats, such as soils during periods of low water activity. In some cases, conditions for growth of the microorganisms may not have been met. In other cases, the microorganisms may be injured and, therefore, difficult to recover.

Recently, Schut et al. (1993) and Bianchi and Giuliani (1996) were successful in using extinction dilution procedures with water from the environment to grow many of the most numerous heterotrophic marine bacteria. In this process, at the time of collection, the original sample from the habitat is serially diluted several orders of magnitude using autoclaved water from the environment until the highest dilution receives few, if any, organisms. In this manner, the most abundant species (from the highest dilution tubes) can be obtained from the habitat, providing they grow in the medium. Following prolonged incubation in sterile natural water samples, many of the most numerous organisms from the environment were grown on ordinary laboratory media. This approach should be attempted for other habitats as well. In situ hybridization or fluorescent antibody techniques also should be used, if possible, to confirm that the isolated strain indeed comprises a significant part of the original natural community (Aman et al. 1995). Any newer procedures are also available for isolation of fungi which show great potential (Gams 1992; Hall 1996). It should be recognized that methods will need to be developed to cultivate the many dominant microorganisms that can be found in natural communities and are not yet successfully cultured. Therefore, research on new methodologies is not only strongly encouraged, but viewed as critical to microbial discovery and harvest (see Infrastructure Needs).

When new microbial strains are cultivated, they need to be characterized in a polyphasic manner (Colwell 1970, Vandamme et al. 1996), including determination of their phenotypic features (morphology, physiology, biochemistry, and genetics) as well as their 16S/18S rDNA and other sequences used to assess their phylogenetic position. Thus, the terms “polyphasic taxonomy” and “polyphasic systematics” refer to the holistic characterization of a species; when the terms taxonomy and systematics are used in this report, this connotation is implied. Pure cultures are suitable for elucidation of physiologic, metabolic, and morphologic traits and evaluation of the commercial potential of an organism. Illustrative features useful in characterizing the phenotype of bacteria are shown in Table 4.

A. Phase contrast of mixture of *Heliolothrix* (most of the filaments), *Synechococcus* sp. (the rods, some with gas vacuoles at poles), and short filaments of *Pseudanabaena*-like cyanobacterium (with conspicuous constrictions at the cross-walls). (Courtesy of Richard Castenholz)

B. The same view under epifluorescence, showing the red phycocyanin/chlorophyll fluorescence of the *Synechococcus*, the orange phycoerythrin fluorescence of the *Pseudanabaena*, and the lack of visible fluorescence in the *Heliolothrix*, which is expected since the bacteriochlorophyll α which they contain fluoresces in the infra-red. (Courtesy of Richard Castenholz)
The phylogenetic position of newly-isolated strains can be used to identify the closest cultivated known species that can be obtained from culture collections for direct comparison with the new strain. It should be noted that, although the 16S rRNA sequence has provided a powerful means to organize the microbial world in a meaningful way, this gene is often too conserved to provide the resolution needed to determine species-level differences for bacteria. However, if the 16S rDNA sequence homology between two bacterial strains is less than 97%, they can be considered to be separate species, based upon definitions of bacterial species in which DNA/DNA reassociation studies have been performed (Stackebrandt and Goebel 1994). If the new bacterial strain is closely related to a known species, then DNA/DNA reassociation tests need to be performed to determine whether it comprises a new species, that is, exhibits <70% reassociation with known species (Wayne et al. 1987).

If a new species can be cultivated, then genetic composition, dormancy characteristics, production of biotechnologically useful products, etc., can be assessed. Cultures are, therefore, useful to both the microbiologist and the biotechnologist. Through characterization of pure cultures, it is also possible to propose hypotheses concerning ecological function (niche) of the organism in the environment. Newer molecular methods should assist in the definition of in situ activities, verification of the importance of features determined in pure culture, and identification of new features. For example, by use of molecular probes to assess the expression of specific genes, it is possible to assess the likelihood that specific activities occur in the habitat (Amann et al. 1995). In this manner, hypotheses regarding the niche of the species can be verified or refuted.

It should be noted here that in some cases it may not be possible to isolate single species because growth may be very tightly coupled with that of another species. In such cases, it may be possible to grow both strains together. Such co-cultures are also appropriate for study in the laboratory.

Because 16S rDNA sequences within a given bacterial species can range from 97 to 100% homology, it is not possible to use this procedure to define a bacterial species. Therefore, collection and evaluation of polyphasic data are encouraged to enhance understanding of microbial species and to adopt a useful assessment of diversity in natural communities.

It is anticipated that, from a polyphasic evaluation, methods will emerge for more direct species-level characterization and identification. At this time, parallel efforts should be undertaken to build economical automated devices to provide such data and to establish electronically available databases (see Infrastructure Needs).

It should also be noted that individual strains or varieties (ecovars) of microorganisms within the same species may have specific functional niches and capabilities. Often it is the subspecific traits of microorganisms that are critical for their survival, function or dominance in a particular environment. Thus, microbial diversity at the functional level goes beyond species diversity to include strain diversity within the assemblages of microorganisms in a habitat.

As new taxa are discovered, especially when they fall into new evolutionary categories, metabolic pathways used for energy generation and biosynthesis should be determined. This aspect of microbial diversity addresses the phenomenal array of physiological and metabolic lifestyles among microorganisms, many of which are still unknown, e.g., the Thermoleophilum group, Aquificales, Verrucomicrobiales, Planctomycetales, barophilic bacteria, and sea ice bacteria, to list a few. Such groups are deserving of study to determine if they have novel biochemical reactions and whether they produce unique and biotechnologically important products. From an ecological perspective, it is important to determine whether the properties expressed by organisms in natural communities differ from properties expressed in pure culture.

### 3.1.1.3. Complementarity Between Cultivation and Non-Cultivation Approaches

Microbiologists need to bring closure to the enumeration anomaly, that is, the inability to cultivate the most numerous bacteria from natural habitats. The use of extinction dilution cultivation procedures, described earlier (Schut et al. 1993; Bianchi and Giulini 1996), combined with identification of environmental phylotypes, discussed above, may offer resolution of this anomaly.
3.1.2. Habitats for Molecular Microbial Inventory

To achieve a reasonably comprehensive census of the diverse forms of microbial life on Earth, a variety of habitats must be studied. At this time, our perception of microbial diversity is rudimentary. Much needs to be done to inventory microbial life in common soil and aquatic habitats, to determine which organisms are responsible for maintenance of these environments, and which are essential for human well-being. Agriculturally important soils and globally important marine habitats are given high priority in this regard. Since microorganisms inhabit many environments that are inhospitable for plants and animals, because of temperature and pH extremes, anoxic conditions, oligotrophy, salinity, low water potential, and organic composition and certain of these habitats, particularly some of the most extreme, such as hot springs, are threatened by societal exploitation, e.g., thermal power generation and/or tourism, it is critical that these be inventoried as soon as possible. Some symbiotic habitats, such as those of threatened plant and animal species, may harbor unique, obligate microbial symbionts that may also become extinct.

Because of these factors, the choice of terrestrial and aquatic habitats for microbial inventory should be made carefully, with consideration given to areas currently under study by botanists and zoologists, so that complete taxonomic inventories of all life forms can be achieved. Thus, the Long Term Ecological Research sites supported by the National Science Foundation offer logical candidate sites for microbial inventory. In addition, the All Taxa Biotic Inventory (ATBI) program underway in Costa Rica is another candidate for site selection (Tiedje 1995), as well as sites under study by museums.

Extreme habitats that are high priority for inventorying are hot springs and deep sea vents, cold habitats such as sea ice microbial communities and polar ponds and lakes, and acidic, alkaline, and anoxic habitats, and those of unusual chemistry, notably sites designated as Superfund Sites by the U.S. Environmental Protection Agency. Recent discoveries of extensive microbial biomass in deep subterranean core samples, including microorganisms which utilize hydrogen gas generated abiotically as a principal energy source (Stevens and McKinley 1995), offer an excellent example of the vast diversity of life awaiting discovery and exploration.

Habitat type will dictate frequency and location of sampling stations. In most natural habitats, temporal changes, including diel and seasonal variations, affect composition and activities of natural microbial communities. Thus, to achieve comprehensive inventory, frequency of sampling is important. Sampling locations, latitude, and longitude are best determined by satellite position coordinates employing the Global Positioning System (GPS). Depth is best measured at a scale compatible with vertical structure of the community, e.g., approximately mm scale for sediments and microbial mats and m scale for marine water column.

Selected physical, chemical, and biological parameters influencing or influenced by activities of microorganisms should also be measured—temperature, light intensity and wavelength, pH, oxygen concentration, salinity, chemical composition, metal concentrations, presence of other microbial, plant, and animal species in the given environment are examples of parameters. This information comprises a valuable component of the database for microbial exploration and harvesting.

Biogeography of microorganisms is an aspect of microbial ecology that is in the developmental state and is relevant to microbial exploration and harvesting. Assessment of the total number of microbial species on Earth will require an understanding of endemic species of free-living microorganisms. It is necessary to understand the geographic limits of cosmopolitan and endemic microbial species in natural habitats. It is yet to be determined how the diversity of microbial species compares and coordinates with that of plants and animals. For example, are there fewer species and less complex communities at polar and temperate latitudes compared to tropical latitudes? Does the microbial species diversity decrease with increasing altitude? Answers to this class of questions will provide the information that will allow estimates of the total
number of microbial species on Earth. At this time, only ca. 4,000 bacterial species have been described—significantly fewer than the 1% of the one million bacterial species that have been estimated to exist today (Table 1). Similarly, microbiologists have described ca. 72,000 species of fungi which compares with estimates of 1.5 million species believed to exist on Earth at this time (Hawksworth et al. 1995). Biogeographic studies of free-living species and symbiont diversity will permit more precise estimates of microbial diversity.

In parallel with estimating the number of microbial species, a focus on the process of speciation which occurs in the natural habitats of microorganisms is necessary. If microbial diversity is to be understood, it is essential that microbial speciation, which ultimately determines the variety and abundance of species on Earth, be elucidated. Presumably, each species has its own niche, thus it is paramount to relate specific types of microorganisms to their particular niche. Studies directed toward elucidating the species/niche concept will be vital to a microbial inventory and harvest.

The diversity of physiological activities in natural habitats is due largely to microbial life resident therein. However, in situ function at other than the community level is difficult to resolve at the microbial level. It is necessary to understand action and interaction of populations contributing to processes at the gross scale employing autecological methods. The presence and variety of species in a given habitat must be linked with evolution and ecological activity, as stated above. This will require in-depth, intensive sampling at specific, selected habitats and micro-habitats to discover underlying principles and driving forces accounting for the existence and evolution of individual species in relation to the environment. Such focused studies, best carried out by a team of researchers examining specific populations in a few selected communities, are strongly recommended. Microbial ecologists will need to develop or adapt technologies for in situ activity measurements to achieve this objective, including fluorescence-based physiological probes, nucleic acid probes, advanced microscopy, and microelectrode applications.

Chemistry-based biomarker methods can provide quantitative analyses of viable biomass, community composition, and nutritional/physiological status. In this latter instance, lipid and phospholipid analyses of community samples have been found to be useful as a means of assessing the biomass of microbial communities and of given microbial groups with signature lipids (White 1995).

In many microbial ecosystems, the functionally active unit is not a single species or population but a consortium of two or more types of organisms living in close symbiotic association. For consortia, it is important, nevertheless, to identify individual types of microorganisms, where possible, so that contributions of each to a given process and identification of the underlying factors which underlie their association can be effected.

Selection of a specific habitat for analysis should be driven by hypotheses relevant to forces determining composition of microbial communities. Two such forces include biotic and abiotic resources selective for the extant community. These features, in conjunction with species dispersal, are important in determining how microbial diversity patterns throughout the Earth are formed. Community studies will provide the opportunity to coordinate concurrent biodiversity studies at the macrobiological level in the same habitats. This will enhance the potential of integrating findings with other inadequately characterized microbiota, viz., nematodes, rotifers, and tardigrades, as well as the plants and animals. This effort can take advantage of existing well-characterized sites, such as the National Science Foundation funded Long Term Ecological Research (LTER) sites, Smithsonian Institution study sites, Man and the Biosphere Programme (MAB), and the All Taxa Biotic Inventory (ATBI) underway in Costa Rica.
Sequencing the Genome of At Least One Species of Each of the Major Phyla of Microbial Life as a Major Objective of Microbial Exploration and Harvesting

The goal of this component of microbial exploration and harvesting is to sequence the complete genome of at least one species from each of the microbial phyla (see Table 5 and Figure 1). Some of the phyla listed include microorganisms either already sequenced or in the process of being sequenced. There are many advantages for one species from each of the remaining phyla being sequenced. Sequencing of eukaryotic microbial genomes will also be informative and useful. The genome of only one eukaryotic genome, that of a yeast, has been sequenced. Since many of the eukaryotic microorganisms have very large genomes and some may be as expensive to sequence as the human genome, those with small genomes are recommended for sequencing. Some candidates include *Giardia*, containing ca. 12 megabases and selected microsporidia, a genome of ca. six megabases. These types of microorganisms appear to have diverged at an ancient time from the eukaryote lineage, before acquisition of mitochondria and chloroplasts. Consequently, they offer a view of the eukaryotic genome before organelle acquisition.

The sequence data will provide the information necessary to understand evolution and interrelatedness of all forms of life on Earth. From the standpoint of evolution of humans and animals, sequencing the genomes of microorganisms will yield molecular information relevant to the nature of ancestors of these organisms with a nervous system and more complete understanding of sensory perception and other important, complex biological processes in plants and animals. Information derived from microbial genome sequences will aid in understanding those genetic features of importance in infectious diseases, food and dairy microbiology, agriculture, biotechnology, and many other applications. The 16S rRNA sequence data with phenetic data, as described above, will provide a powerful database.

4.1. Infrastructure

To accomplish the major goals of microbial exploration and harvesting, an underpinning infrastructure must be in place. Infrastructural requirements fall into several categories, as follows: education; instrumentation; national and regional researcher centers of excellence; database; and genetic resources.

4.1.1. Education

It is fundamental that the public understand a revolution has occurred in biology. New knowledge about the enormous diversity of microorganisms and the importance of this knowledge for the health of the biosphere and to human welfare must be shared with teachers, students, and citizens of all ages. This new awareness in microbiology must be promulgated by scientists at the forefront of research. It is clear that current understanding of the life forms sharing planet Earth is very poor indeed. This new knowledge must be incorporated into biology textbooks to replace out-of-date information concerning microbial diversity. Similarly, exhibits in natural history museums should incorporate illustrations of microbial diversity and its relevance to our daily lives. At the university level, increased funding is critically needed to train
the next generation of microbial biologists, especially systematists. Programs should be established or expanded to encourage predoctoral and postdoctoral students to investigate biological properties of heretofore poorly studied microorganisms.

General Public and K-12 Level

The general public needs to be informed of the exciting new forms of life that have been discovered and about microbial diversity and biotechnology, especially the value of these to everyday lives and activities. Natural history museums, arboretums, conservatories, wildlife parks, and zoos are logical places for public education. Exhibits have been established for microorganisms on a smaller scale in selected parks, e.g., Yellowstone Park, Museum of the Rockies (Landform: Lifeforms exhibit), and Archaean Hall at the Smithsonian Institution.

However, in general, microbial exhibits are rare. Culture collections, the microbial counterpart of zoos and natural history museums, typically do not provide exhibits in their facilities. Facilities for displaying and explaining the importance of microbial diversity should be available to the public and should be incorporated into existing museums, national parks, and monuments, as well as new facilities in association with national culture collections. Pamphlets and books on microbial diversity and evolution need to be made for the general public.

Television is a very effective means of reaching the public. Natural history programs comprise part of the regular programming of the Public Broadcasting System and Discovery channels. However, very little information is provided about microorganisms; such programs need to be developed. Potential topics include: the search for novel microorganisms in unusual and common habitats; the history of the hunt for microbial agents causing human disease by Louis Pasteur, Robert Koch, and others; and the discovery of antibiotics and their impact in controlling infectious disease. Other possibilities include programs on Sergei Winogradsky, who discovered chemolithotrophy, and Martinus Beijerinck, who studied bacteria carrying out nitrogen cycling.

The World Wide Web and other Internet services can also be used effectively to reach the general public. Home pages and news groups established to take advantage of current and emerging Internet technologies, including images, video clips, animations, three-dimensional renderings, and virtual reality “microbial” worlds are effective means of public education. The usefulness of “Weekly Reader”-type newsletters that reach students of various backgrounds is recognized, as are World Wide Web and CD-ROM features. K-12 students also need “hands on” projects that generate data. The Jason project and GLOBE offer useful models.

University Level

- Microbial Physiologists

The most critical need for microbial diversity research to be met is the training of systematic and evolutionary microbiologists, especially those who study taxonomy, physiology, and autoecology of microorganisms. A survey by the National Science Foundation (Edwards, David, and Nerling, The Systematics Community [Museum of Natural History, Lawrence, Kansas, 1985]; Gaston and May, 1996) indicated that 60% of U.S. systematic and evolutionary biologists study animals, 30% study plants, 5% investigate fossils, and a mere 2% work with microorganisms! Indeed, of the 42,000 members of the American Society for Microbiology, only 160 (less than 0.5%) have a primary interest in microbial systematics. A similar situation exists in mycology, in which declining numbers of systematic mycologists are being trained (Burdsall 1990). Many of the currently active microbial systematists are senior scientists approaching retirement. Thus, despite the fact that most of the unknown biological diversity on Earth is microbial, there are far too few scientists to undertake the important and exciting task of microbial discovery and harvesting. Increased support is critical for educating young scientists who will characterize and classify the microorganisms extant on Earth.

- Microbial Diversity Special Courses

Special courses on microbial diversity must be supported. Those in place need to be continued, and new courses developed. Successful summer courses are currently being taught at Woods Hole (Microbial Diversity and Molecular
Evolution), Ohio State University, and the University of Georgia. Courses on microbial diversity need to be developed for mycology and protistan biology.

4.1.2. Instrumentation

Microbial exploration and harvesting will require collecting samples that will yield large numbers of novel microorganisms. A major obstacle in comparing large collections of organisms, one to another, is the lack of rapid methods and instrumentation. Currently available instrumentation, including in situ PCR, DNA sequencing and phylogenetic analyses, whole cell fatty acid analyses, gas chromatography-mass spectrometry, high throughput chemotaxonomic methods, image analysis (for protozoal identification), laser trapping and other procedures for isolation of new species, etc., are required in taxonomic research. New methods and instrumentation to process large numbers of samples rapidly and in miniaturized format and providing robust identification of species, are sorely needed. Therefore, it is recommended that new methods and instruments that allow a battery of tests of hundreds of strains simultaneously and yielding results that are directly comparable be developed. An equally urgent need exists for improved methods for analysis of data derived from such studies, as well as for presentation of data employing effective and efficient visualization. Instruments for collection of samples from various environments, assessment of physiological activities and microbial distributions in natural communities, technology and equipment for polymerase chain reaction (PCR), molecular sequencing and phylogenetic analysis, DNA/DNA reassociation, cultivation, and phenotypic characterization of strains are fundamental to microbial discovery and exploration.

4.1.3. Specialized National and Regional Microbial Diversity Research Centers of Excellence

To train scientists in evolution and systematic microbiology, national and regional research centers are recommended. Students can be taught procedures and rules of microbial taxonomy by experts and specialists and receive training in techniques and use of instruments for sequencing and phylogenetic analysis of DNA, RNA, and protein, specialized chemical and biomarker analysis (lipids, carbohydrates, proteins), microscopy, and analytical instrumentation (spectroscopy, etc.) for chemotaxonomy, and physiological methods. Such centers can serve as training sites for scientists from other countries, including developing countries with microbiological resources of biotechnological interest.

It is recommended that centers, funded competitively, be established near habitats selected for specific environmental inventory. For example, a center focused on hyperthermophilic bacteria can be established at a geographical location to take advantage of hyperthermophilic habitats. Similarly, one or more centers for soil or sediment microbiology, regional centers for marine microbiology, and a national or several regional centers for training bacterial taxonomists and eukaryotic microbial taxonomists can be a highly effective mechanism for achieving cost-effective investment in microbial exploration and harvesting. At these centers, students would learn fundamental principles and issues of microbial taxonomy and phylogeny and specific techniques, e.g., 16S/18S rDNA sequencing, DNA/DNA reassociation, methods for isolation of newly discovered taxa, and phenotypic analysis, including cellular lipid analysis.

4.1.4 Databases

Efficient recall of the body of existing microbial knowledge is essential for biodiversity studies, as well as for practical uses, such as quality control, diagnosis, and pharmaceutical discovery. The 16S rRNA (in the Ribosome Database Project, RDP) and other microbial databases in the U.S., including the USDA yeast database and a ribosomal database at the University of Texas, are currently available. A significant need exists to curate taxonomic, phylogenetic, biochemical, and ecological data in electronic format and to develop the means to integrate these and new databases to address complex queries seamlessly. Important goals are to develop new electronic databases, enhance existing databases, and integrate all microbiological and related
databases (Green 1994), to be publicly available in a simple, accessible form.

To achieve the goals of microbial exploration and harvesting, information on new discoveries must be made available in databases, especially for rapid comparison with existing strain data and related information. Microbial databases, including those for bacteria and eukaryotic microorganisms, are at the conceptual stage, with only the Ribosome D database Project having served scientists for a number of years. An integrated microbial database that includes the Ribosome D database and Phenotype D database and on-line, Internet databases for characterizing, identifying, and classifying microorganisms would be extremely valuable.

Bacterial databases in the conceptual or developmental stages include the following:

The Ribosomal rRNA Database Project (RDP) and Phenotypic Database Project (PDP)

Automation of updating and annotations and development of new ways to generate and assess phylogenetic trees, secondary structure program, new services (e.g., probe and primer design), and quality control against such problems as chimera recognition, as well as sequencing and adding data for unsequenced type strains and resolving problems with nomenclature, including synonymy, are tasks recommended to enhance the RDP.

A PDP has been proposed by Bergey's Manual Trust, which produces the major taxonomic treatise in bacteriology. The PDP entails development of a program for phenotypic data for Eubacteria and Archaea, to be made available in a single database readily accessible and continually updated. A fatty acid methyl ester (FAME) database also needs to be included.

Integrated Microbial Database

An on-line integrated microbial database that organizes and links all microbial databases with databases already on, or to be added to, the Internet, e.g., Protein, Metabolic, and GenBank databases (Tiedje et al. 1996) is recommended. Other databases that should be developed include habitat type, ARDRA (Amplified Ribosomal DNA Restriction Analysis) information, and morphological images. Such databases are needed for other microbial groups, including the fungi, protists, and photosynthetic microbial lineages, i.e., microalgae. In addition, the USDA ARS National Fungi Collection databases should be expanded to include whole organism data.

4.1.5 Genetic Resource Collections

Microbial culture collections serve a vital role for all microbiology. Like particle accelerators in physics, culture collections provide the basic research tools for microbiologists. Not only do they contain key microorganisms, but they are the principal reservoirs of all the diverse genetic and biochemical resources of microorganisms available in culture.

In the United States there are several important microbial collections that are members of the United States Federation of Culture Collections. These include the American Type Culture Collection (ATCC) and the United States Department of Agriculture ARS Collection. The ATCC and certain other collections in the United States receive only partial support from the federal government. In fact, the ATCC is a private non-profit institution. In contrast, both the Japanese and German national culture collections are fully supported by their respective federal governments. Similarly, the Netherlands Royal Academy funds 85% of the cost of the Netherlands Culture Collections (NCC) which arguably contains the world's premier fungal collection. Furthermore, active basic research programs are supported in many of the other national culture collections, whereas too few resources are available for a significant level of research activity in U.S. collections. For the sake of this argument, it is useful to compare the situation of the ATCC with that of governmentally funded national museums, such as the Smithsonian Institution, which have resources for research activities. When new microorganisms are described and named, they should be deposited in the ATCC or other collections, thereby expanding accessibility. Indeed, national and international patent laws require patent strains be deposited in permanent collections. In view of international trade competition, it is important that national collections in the United States receive long-term,
stable support from the federal government to handle activities associated with microbial exploration and harvesting.

The 16S or 18S rDNA of all microorganisms deposited in culture collections should be sequenced, including not only those newly accessioned but also those already resident in collections. Serious consideration should also be given to establishing a DNA collection for all microorganisms, specifically bacteria, to expedite DNA/DNA reassociation analyses.

In addition to preserving type strains of microbial species, consortia and perhaps even entire natural microbial community samples should be preserved, especially those of endangered habitats. This can be accomplished by conservation under liquid nitrogen. Culture collections in the United States represent a genetic resource for scientists and industry. Among microbial strains deposited in culture collections are species that serve as “gold standard” for comparison with newly discovered organisms. National collections should serve as learning discovery centers for exploring and understanding the natural history of microorganisms for the public, as is done in natural history museums housing animal and plant collections. Educating the public to appreciate the microbial world, especially in understanding the importance of microorganisms for human health and in the evolution and functioning of the biosphere, can, thereby, be effected.

Cells of *Vibrio cholerae* O1 stained with fluorescence iosthiocyanate labeled monoclonal antibody (Cholera DFA™, New Horizon Diagnostics, Columbia, MD). (Courtesy of Anwar Huq and Rita R. Colwell of the University of Maryland Biotechnology Institute, College Park, MD)
Societal Benefits of Microbial Exploration and Harvesting

Microorganisms have provided many economically valuable products and processes used every day by all citizens. With the advent of modern biotechnological tools and processes, potential applications of microorganisms are expected to increase significantly. Exploration of microbial diversity will not only be a voyage into Earth's biosphere that will lead to discovery of new and unusual organisms, but also a means of discovering new products and technologies for agriculture, bioremediation, medicine, pharmaceutical industries, and biotechnology.

The anticipated impact on the world economy of microbial exploration and harvesting is expected to be significant and long-lasting. For example, cephalosporin antibiotics alone have a worldwide annual wholesale market value of approximately $8 billion. Other, new billion dollar biotechnologies have resulted from applications using microorganisms. In fact, one of the major contributions to the explosive growth of the biotechnology industry is the polymerase chain reaction (PCR), which relies on the thermophilic bacterial enzyme, Taq polymerase, that functions at high temperatures close to the boiling point of water.

Harvesting microorganisms does not damage natural environments or adversely affect ecosystems since only small amounts of material are needed. When desirable features have been identified in novel microorganisms, they can be grown in industrial fermentors for commercial production. Microorganisms are not exploited to perform industrial, agricultural, and commercial activities; they perform these activities to sustain their own livelihood, and it is utilization of these activities for application to the betterment of the human condition that is envisioned. Table 2 lists examples of present and predicted applications of microbial biotechnological tools and processes that could potentially be derived from microbial exploration. Even if only a single antibiotic comparable to erythromycin or penicillin results from this program, millions of lives could be saved and a commercial product worth hundreds of millions of dollars per year could result.
Policy Recommendations

• Inventories of living organisms in the U.S. are well behind those of other countries. In addition, microbiology worldwide lags far behind the plant and animal sciences in biotic inventories. If microbial exploration is not undertaken soon, there will be a negative impact on both the scientific and industrial capabilities of the country. Therefore, it is recommended that federal agencies in the United States provide financial support for microbial exploration and harvesting as soon as practical.

• Microbial diversity research is especially important because microbiological resources are commercially beneficial to those countries that can identify and develop them. Because research in microbial diversity will impact all aspects of society, primary funding for microbial exploration and harvesting should come from federal agencies. Industry is encouraged to participate in this effort, in particular pharmaceutical and biotechnology companies.

• Microbial diversity fall within the purview of many federal agencies. Thus, an interagency plan should be developed to support major aspects of microbial exploration and harvesting, including the National Science Foundation, Department of Energy, National Institutes of Health, Environmental Protection Agency, National Oceanic and Atmospheric Administration, Department of Defense, Centers for Disease Control and Prevention, U.S. Geological Survey, U.S. Department of Agriculture, and National Aeronautics and Space Administration.

• Like all major, new scientific initiatives, microbial exploration and harvesting should be undertaken for sufficient duration to ensure return on the research investment; a 10-year initiative would be appropriate.

• Several international research efforts in biological diversity, such as DIVERSITAS and SCOPE, and European and Asian efforts, are already underway. The U.S. should join with these efforts where practical, initiate new international efforts as needed, and foster international cooperation and collaboration in this area, especially in microbial diversity. The potential value of microbial diversity provides compelling justification for launching a major national program in microbial exploration and harvesting.
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Figure 1. The universal tree of life based on phylogenetic analysis of 16S and 18S rDNA of various microbial groups and plants and animals. Note the extraordinary variety of microbial groups (herein termed Kingdoms) in comparison with the plant and animal Kingdom branches. Note also that the bacteria are separated into two phylogenetic groups termed Domains, called the Bacteria and Archaea, and the other microbial groups are all grouped with the Plant and Animal Kingdoms in the Domain Eucarya. Adapted from Carl Woese (1994).
Table 1. Approximate Numbers of Species in Major Taxonomic Groups (in thousands) and Percent Known

<table>
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<th>Percent</th>
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<tr>
<td><strong>Animals</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nematodes</td>
<td>25</td>
<td>400</td>
</tr>
<tr>
<td>Crustaceans</td>
<td>40</td>
<td>150</td>
</tr>
<tr>
<td>Insects</td>
<td>950</td>
<td>8000</td>
</tr>
<tr>
<td>Vertebrates</td>
<td>45</td>
<td>50</td>
</tr>
</tbody>
</table>

1 Adapted from Heywood, 1995.

2 These categories are based on traditional definitions.
Table 2. Current and Anticipated Benefits of Microbial Exploration and Harvesting

A. Current Successful Applications of Microbial Biotechnology that would be Enhanced

1. Industrial fermentations
2. Industrial enzymes—current U. S. production is approximately $1 billion/yr.
3. Bioactive compounds—antibiotics, antiinflammatory drugs, immune suppressors, lipid regulating agents (e.g., mevinolin), biopesticides (e.g., avermectins, milbemycins), genetically engineered vaccines (e.g., hepatitis A, B), growth promoters (e.g., certain antibiotics). U. S. production is $35 billion/yr.
4. Food and dairy industry processes and products
5. Pulp and paper effluent treatment
6. Water and wastewater treatment
7. Bioplastics

B. Anticipated Products, Applications, and Areas Expected to Benefit

1. Enzymes for biotechnology industry: polymerases, ligases, alkaline phosphatases, proteases
2. Biocatalysts (enzymes)—e.g., robust catalysts that function at high or low temperatures; chiral synthesis; high and low pH, high salt, activity in organic solvents
3. Bioactive compounds such as antibiotics and anti-inflammatory compounds from soil, endophytic and epiphytic fungi and bacteria, coprophilic and litter-inhabiting fungi and bacteria, or associated with marine macroorganisms
4. Sustainable agriculture and forestry
   a. Bacterial strains as pesticide; for example *B. thuringiensis*
   b. Nitrogen fixation
   c. Rhizoremediation, plant growth-promoting rhizobacteria
   d. Robiotics, animal health promoting substances and microorganisms
   e. Plant genetic engineering with *Agrobacterium*
   f. Biocontrol agents
   g. Mycorrhizae
5. Bioremediation of hazardous waste sites, and biological treatment of industrial waste streams
6. Recycling
7. Biomimetics: exopolymers, dispersants for ceramics, adhesives, emulsifiers
8. Biocomputers and switches
9. Biorestoration and bioconservation:
   a. Green technologies
   b. Mine reclamation
   c. Microorganisms important to conservation of plants and animals in endangered habitats
10. Human health
    a. Diagnostics and public health
    b. Water and wastewater treatment methods (a major contribution microbiology can make to public health worldwide)
    c. Emerging infections
    d. New antibiotics to combat antibiotic resistant pathogenic organisms
    e. Air quality in buildings
11. Impact on global climate: trace gases (carbon dioxide, methane, dimethyl sulfide)
12. Microbial production of fuels: hydrogen, biodiesel, alcohol
13. New discoveries that expand our understanding of the diversity of life and the impact of microorganisms on the biosphere
Table 3. Environmental Extremes under which Microbial Life Occurs on Earth

<table>
<thead>
<tr>
<th>Environmental Parameter</th>
<th>Microbial Group</th>
<th>Habitat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature extremes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low: &lt;1°C</td>
<td>Psychrophilic Eubacteria</td>
<td>Polar sea ice communities</td>
</tr>
<tr>
<td>High: &gt;110°C</td>
<td>Hyperthermophilic Archaea</td>
<td>Hot springs, marine hydrothermal vents</td>
</tr>
<tr>
<td>pH extremes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low: ≤1</td>
<td>Acidophilic Eubacteria, Archaea</td>
<td>Sulfur springs, acid mine drainage</td>
</tr>
<tr>
<td>High: &gt;12</td>
<td>Alkaliphilic Eubacteria, Archaea</td>
<td>Soda lakes, desert soils</td>
</tr>
<tr>
<td>Absence of Oxygen</td>
<td>Anaerobic fungi, protozoa, Eubacteria, and Archaea</td>
<td>Sediments, animal intestinal tracts</td>
</tr>
<tr>
<td>Saturated salts</td>
<td>Extreme halophilic Archaea</td>
<td>Salt lakes, brines</td>
</tr>
<tr>
<td>Low Water Activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Relative Humidity)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.55 (55%)</td>
<td>Xerophylic fungi</td>
<td>Deserts, saps, brines</td>
</tr>
<tr>
<td>High Hydrostatic Pressure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;1,000 atmospheres</td>
<td>Barophilic bacteria</td>
<td>Deep sea</td>
</tr>
<tr>
<td>High Radioactivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Radioduric bacteria, e.g. Deinococcus group</td>
<td>Radioactive sites, soils etc.</td>
</tr>
</tbody>
</table>

1 It is noteworthy that microorganisms are also known that can live in habitats that have combinations of extreme conditions, such as high temperature and low pH (acidothermophiles) and high temperature and high pH (alkalithermophiles), as well as others such as halophilic thermophiles, psychrophilic barophiles, etc.
Table 4. Typical Features Used for the Description of Bacteria

Morphology
- Cell shape
- Cell size (diameter, length)
- Motility
- Flagellation
- Type of cell division
- Cell differentiation
- Internal or external structures (endospores, gas vesicles, etc.)
- Gram stain
- Ultrastructure

Chemical composition and molecular analyses
- Color of cell suspension
- Pigments
- Reserve materials
- DNA base composition
- 16S rRNA sequence
- DNA/DNA reassociation for species determination
- Whole cell fatty acid composition

Physiology
- Growth medium
- Temperature range and optimum
- pH range and optimum
- Phototrophic or lithotrophic growth
- Vitamin requirements
- List of carbon sources used for growth
- List of nitrogen sources used for growth
- Relation to oxygen
- Modes of energy generation
  - electron donors: either organic or inorganic
  - electron acceptors: oxygen, nitrate, sulfate, carbon dioxide, iron oxides, etc
- Extracellular enzymes
  - amylase
  - lipase
  - gelatinase
  - cellulase
  - xylanase
- Other enzymes
  - catalase
  - oxidase
- Glucose fermentation
- Denitrification
Table 5. List of Major Microbial Phylogenetic Groups or Kingdoms

**Eubacterial (Bacterial) Kingdoms**

Proteobacteria*
  - alpha
  - beta
  - gamma* (E. coli; Haemophilus influenzae)
  - delta
  - epsilon
Green sulfur bacteria
Green filamentous bacteria
Cyanobacteria* (Synechococcus)
Gram positive bacteria* (Staphylococcus aureus; Bacillus subtilis.)
Planctomycetes group
Chlamydia
Cytophaga/Bacteroides
Spirochetes* (Treponema sp.)
Deinococcaceae/Thermus (Deinococcus sp.)
Other thermophilic Eubacteria (Aquifex,* Thermomicrobium, etc.)

**Archea**

Methanogens*
Extreme halophiles*
Hyperthermophiles*

**Eucarya**

Fungi
  - Chytridiomycetes
  - Zygomycetes
  - Basidiomycetes
  - Ascomycetes* (Saccharomyces cerevisiae)
Protists
  - Myxomycetes (slime molds)
  - Alveolates
  - Chromophytes (Stramenopiles)
  - Parabasiliids
  - Rhizopods
  - Cryptophytes
  - Diplomonads
  - Trichomonads
  - Microsporidia
  - Actinopods

* Refers to taxa in which the genome of at least one species has been or is being sequenced