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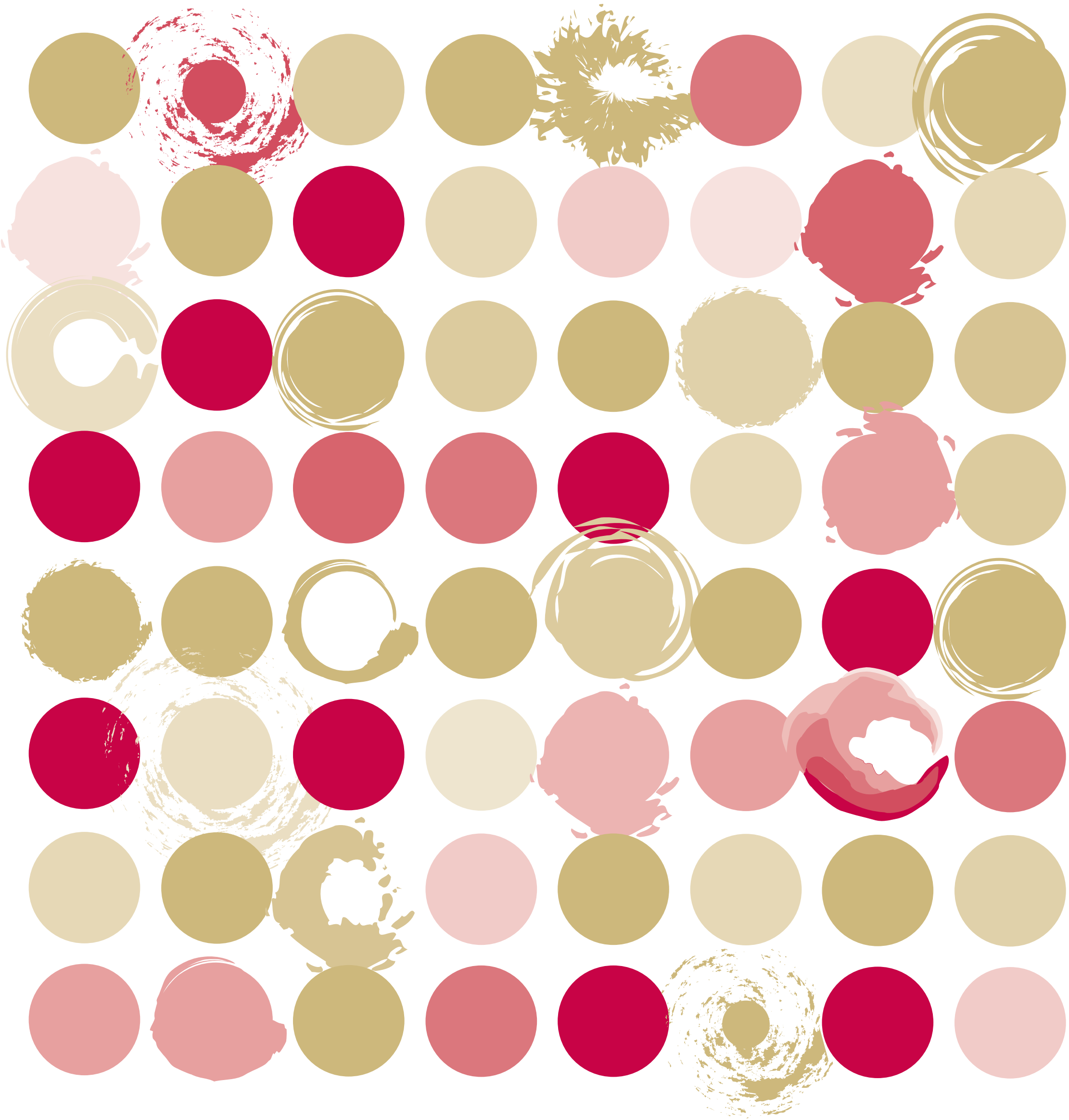

MICROBIAL COMMUNITIES:

From Life Apart to Life Together

AMERICAN
SOCIETY FOR
MICROBIOLOGY

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This report is based on a colloquium, "Microbial Communities: Advantages of Multicellular Cooperation," sponsored by the American Academy of Microbiology held May 3-5, 2002, in Tucson, Arizona.

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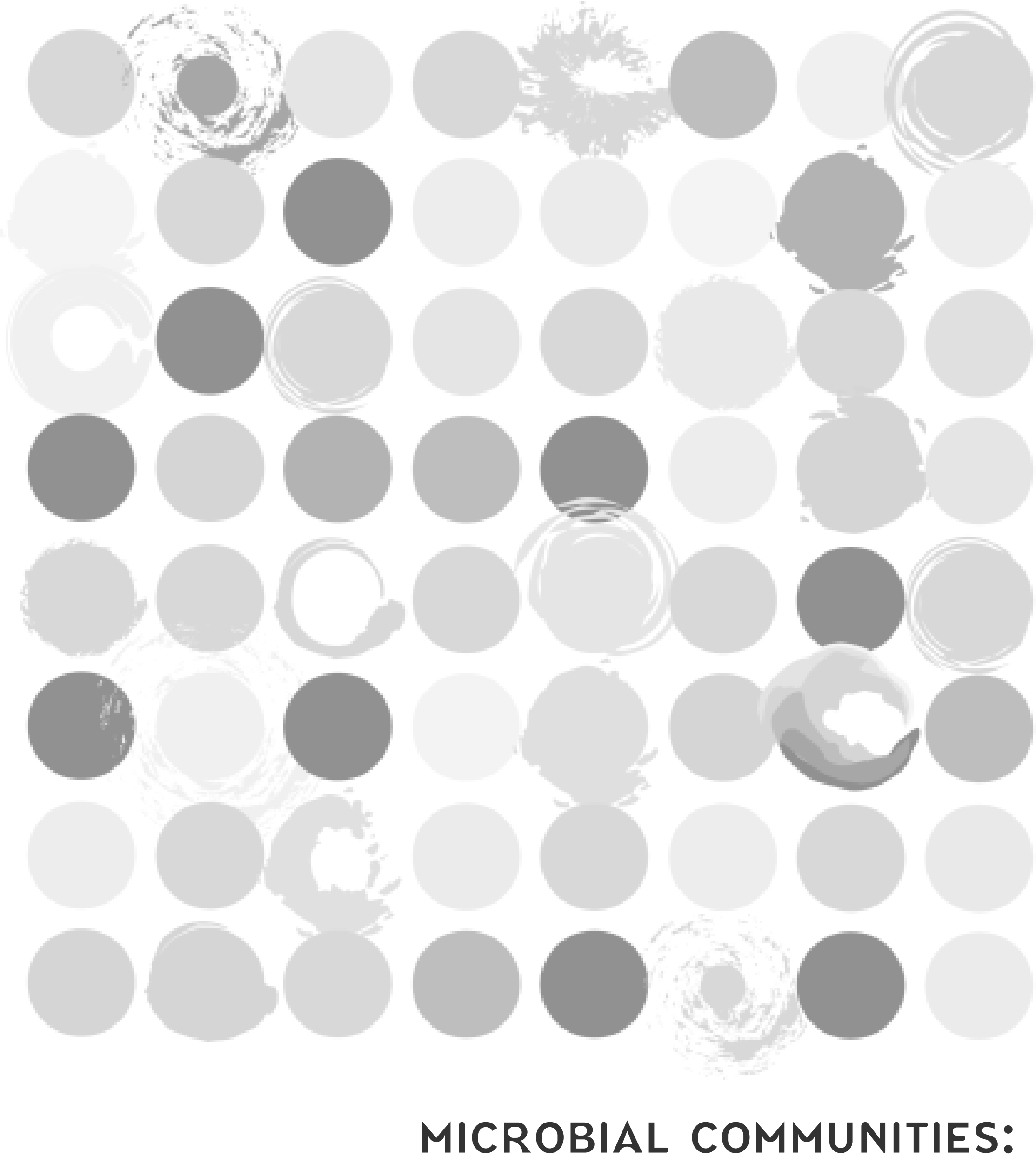

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1.0 Executive Summary

A colloquium was convened in Tucson, Arizona, May 3-5, 2002, by the American Academy of Microbiology to discuss issues surrounding microbial communities and their role in human health, industrial processes, and ecological functions. Discussions centered around the importance of microbial community functions to human and environmental concerns, the status of current research findings in the field, the technologies available for investigating communities, and educational and collaboration needs. The colloquium attendees agreed on a number of recommendations with respect to research, education, and collaboration.

The size of microbes belies the massive impacts they have across the globe. Some microbial communities are enormous. Their contributions are diverse, and their impacts can be felt on every scale—from subtle human infections to the treatment of chemical contamination to the cycling of the elements that are most critical to maintaining life on the planet.

Research has shed light on some of the phenomena surrounding microbial communities, providing clues about their stability, development, and the mechanisms that govern the locations of individual members. However, much work lies ahead. The contribution of unculturable microbes to the functioning of many microbial communities remains a vast, unanswered question. Antibiotic resistance is often enhanced in microbial communities that have organized into biofilms. The problems associated with this resistance must be resolved. Moreover, the role of intercellular signaling and contact-dependent gene regulation is intriguing and should be investigated further in order to better understand the ability of communities to act as an integrated unit. Development of a few appropriate model communities, which would help researchers to address these and other questions, is highly recommended.

Microbial communities operate on every scale and in countless different environments. As a result, research and education efforts need to incorporate contributions of scientists from many different scientific disciplines; from molecular biology to oceanography, the participation of professionals in all relevant fields should be fostered and valued. Improving the public's grasp of the issues surrounding microbial communities through the popular press, the Internet, and other outlets is also encouraged.

2.0 Introduction

Right now, inside the water pipes in your home, thick accumulations of organisms are living off the meager nutrients they glean from tap water. Though mostly harmless to human health, these accumulations are difficult to remove and nearly impossible to prevent, and they foul waterlines and corrode countless miles of water pipes every year. After tap water leaves the faucet and drains down the sink, it follows the waste pipes from your home, under the streets to the treatment facility, and arrives at a biological reactor, seething and gurgling as it digests every manner of substance. Human and animal waste, soaps, food, solvents, and more are degraded and mineralized by the organisms that cling to the surfaces of the tiny reactor particles designed to support them.

After treatment, wastewater is released from the facility into a nearby watercourse, where communities of microscopic organisms thrive in the water column, on the surfaces of rocks, and on sediment particles, exploiting and exchanging the remaining nutrients from the effluent and from naturally occurring sources. As the water continues to flow downstream, it joins larger rivers and eventually meets the oceans, home to vast communities of suspended microorganisms that process all the materials necessary for life. Unfathomable quantities of carbon, oxygen, nitrogen, and other elements pass through the cells of the ocean plankton every day, continually contributing to global atmospheric gases and buffering the chemistry of the world's seas.

The films on the insides of household water pipes, the roiling tank of bioreactor grains, the microscopic assemblies of organisms that extract nutrients from streams, and the ocean plankton responsible for global cycling of nutrients are all examples of microbial communities, groups of microorganisms that exist together, frequently organizing and functioning as a single unit. Their impacts extend beyond the home and beyond water treatment facilities to impact almost every aspect of human and environmental health.

A colloquium was convened in Tucson, Arizona, May 3-5, 2002, to discuss microbial communities, their impacts on humans and the environment, and directions for future efforts in the field. The core topic of the colloquium, "microbial communities," is a seemingly straightforward term, but it is a source of some debate. In a general sense of the phrase, a microbial community is any group of microorganisms that have different functions and activities from one another. A liberal definition identifies microbial communities simply as assemblages of microorganisms, whether single species or multiple species, existing in the same place. This description echoes the macroecological concept of plant and animal communities—assemblages of single species or multiple species of organisms. Scientists tend to agree that many, if not most, communities are composed of more than one species, but it remains useful to include single species assemblages within the definition of "community." Another attribute of many microbial communities is interaction among the member organisms. Although microbial communities often exhibit collaborative activities and complex structure, these are not defining characteristics. The broadest definition, "microorganisms that exist in a defined place," is used in this report.

This report summarizes the discussions and recommendations of the colloquium participants. Discussions centered around the roles of microbial communities in human health and in ecological and industrial processes, the current extent of our knowledge in the field, the techniques currently available for studying these communities, and recommendations for future efforts in research, collaboration, and education. The recommendations included in this report have significance for researchers investigating microbial communities and aligned topics, scientific funding agencies, the popular press, and other interested groups.

3.0 The Impacts of Microbial Communities on Humans and the Environment

The impacts of microbial communities can be felt in every aspect of life and in every corner of the globe. From the evolution of higher organisms to the scourge of antibiotic resistance to the degradation of hazardous wastes, microbial communities are capable of radical impacts on human health and the health of our environment.

In perhaps what could be called the “original” example of microbial communities, the origins of eukaryotes may be attributable to close associations between different kinds of microbial cells. It is well accepted by most evolutionary microbiologists that the eukaryotic cell arose from a close association between different types of single-celled organisms, eventually leading to the engulfment of one microbe by another, leading to endosymbiosis. According to this theory, endosymbiotic relationships arose between one type of single-celled host organism and a series of other, different species of organisms that imparted various capabilities. Mitochondria, which allow eukaryotes to generate chemical energy efficiently by using cross-membrane gradients within the cell to drive the energy production, are thought to have arisen in this way. There is also compelling evidence that the chloroplasts that allow plants to utilize light energy arose from an association between a eukaryote-like cell and a photosynthetic symbiont that found hospitable conditions inside the eukaryote. Hence, microbial communities can be seen as the incubator in which the ancestors of all higher life on the planet were born.

Among non-scientists, the topic of microbes usually goes hand in hand with discussions of disease. Although disease-causing microbes constitute a tiny fraction of all microorganisms, the impacts of these bad characters are impossible to ignore. Microbial communities are responsible for a variety of common afflictions, but conditions caused by biofilms are often the most difficult to treat. Microbial communities exist in countless different environments, including those that exist on solid surfaces. In this type of habitat, a microbial community is called a biofilm. It is important to note that, as assemblages of microbes on a surface, all biofilms are microbial communities, but not all microbial communities are biofilms. Liquid and semi-solid environments also play host to thriving microbial communities.

Diseases caused by biofilms include gingivitis, an infection of the lining of the gums that turns aggressive and destructive under the appropriate conditions. Microbial communities can be responsible for endocarditis, an infection of the lining of the chambers of the heart, and persistent otitis media, a common condition in which the middle ear becomes infected by a refractory biofilm community. Biofilms can develop on almost any solid surface that is implanted in the human body, posing considerable risk in any procedure that requires the use of catheters or device implants. The diseases for which biofilm microbial communities are responsible often exhibit heightened resistance to antimicrobial treatment and to host defenses.

Microbial communities are also responsible for many processes of industrial and environmental significance. Corrosion brought about by microbial communities costs industry billions of dollars every year. Especially impacted are those applications in which water is in continuous contact with a solid surface, as in cooling systems or in marine trades. This power of microbial communities to degrade solid and dissolved substances is harnessed in wastewater treatment bioreactors, which break down sewage into carbon dioxide, which is released, and methane, which can be harvested for producing energy. Microbial degradation is also put to use in the biodegradation of anthropogenic chemicals to harmless products via processes known as bioremediation. Some substances, like PCBs, are not readily degraded by single microbial species, but instead require the coordinated action of multi-species communities to carry out the various steps of the process.

Microbial communities are also responsible for many of the processes carried out in the global cycling of water, oxygen, carbon, hydrogen, sulfur, and other critical substances. Water and the elements that microbial communities interchange are fundamental to the continued existence of life on earth. Microbial contributions to the processes that move these substances through the biosphere are so great that they impact weather patterns and affect the processes that buffer and contribute to global warming.

Evolution, disease, corrosion, degradation, bioremediation, and global cycling are only a few of the many thousands of ways that microbial communities impact our lives. Microbes are ubiquitous across the surface of the earth, and every day they are found to affect human health and the environment in new and unforeseen ways. As with other basic research, a detailed understanding of microbial communities will provide insights that can drive practical applications. Research into microbial communities will enable development of new techniques for controlling microbial growth, generating new natural products including novel pharmaceuticals, improving the safety of medical products and procedures, and optimizing the environmental processes that affect global ecological health. Further insights into these impacts are gained as scientists use traditional and cutting-edge techniques to tease apart the stories behind microbially mediated processes and the communities that drive them.

4.0 Research on Microbial Communities: Early Progress

Microbial communities can be found in every corner of the globe, from the permafrost soils of the arctic circle to termite guts in sub-Saharan Africa, and on every scale, from microscopic films on implanted medical devices to the oceans' planktonic communities that breathe the world's gases and cycle its nutrients. Despite this, microbes at the community level are only beginning to be understood by researchers. Inroads have been made in grasping the processes at work in community stability, development, structure, and other areas, and advances continue as researchers investigate these ubiquitous and diverse assemblages.

Fitting Microbial Ecology Into Macroecology Paradigms

A communication gap has developed between microbiologists and ecologists, occasionally growing so wide that some in ecology have claimed that microbial ecology falls short of "true" ecology. However, microbial communities have been found to adhere to the same basic rules of ecology that macro-communities follow, including the maintenance of different levels of organization, temporal progression, and the existence climax communities. However, there are characteristics inherent to microbial communities that are due to the small distances and microenvironments relevant to microbes.

Another issue is the concern that microbes may engage in horizontal genetic exchange within communities. These attributes set microbial communities apart from communities of metazoan organisms and require that general ecological principles be adapted to the particular idioms of microbial ecology.

Resilient and Stable

One of the more fascinating attributes of microbial communities is the paired properties of resiliency and stability they exhibit. Microbial communities are capable of recovering from and adapting to radical habitat alterations by altering community physiology and composition. In this way, they are able to maintain great stability in structure and function over time. Environmental alterations bring about community change by impacting individual components of the community, but the results are manifested at the level of the whole community. It is tempting for microbiologists to attribute community stability to genetic diversity and functional redundancy, but experimental work has not yet proven this principle to hold true in every situation.

Microbial communities share the ability to maintain biological stability, known as homeostasis, with other biological and ecological entities. Microbes within communities are often buffered from changing environmental conditions, but when exposed to conditions that exceed this adaptive tolerance, a microbial community will destabilize. These conditions, which can include nutrient starvation, viral infection, and other environmental insults, can be viewed as catastrophes for the community. Human activities, in particular, can be responsible for destabilization of communities. For example, supplying a diet of grain to cattle has been shown to lead to a lower rumen pH, which alters the gut community and fosters the growth of pathogenic strains like *Escherichia coli* 0157:H7. Oftentimes, a radically different microbial community may take the place of the former under the new conditions.

Development

FROM EARLY AGGREGATES TO MATURE COMMUNITIES

Microbial communities are diverse, and they may be formed by a variety of different "developmental" processes. Initial aggregates that colonize a surface, for instance, may originate from pre-formed aggregates or single cells, and can develop into mature communities through a number of routes, including the recruitment of planktonic members, clonal growth, and surface motility. A progression of colonizers eventually results in a complex, mature community that does not necessarily resemble the original colonizers. Often, the structuring of aggregates will be dictated by opportunities for co-metabolism. In the case of dental plaque, for example, the aggregated cells of the mature community are the product of, but substantially different from, the initial aggregates. Progression and change are not routes followed by all microbial communities, however, and none of the rules of community development are universally applicable.

DEVELOPMENTAL PROGRAMMING

Among eukaryotes, development refers to the specific series of changes an organism undergoes during the passage from the embryonic state to maturity. These are irreversible processes that take the organism from a lower to a higher state of organization, changing its shape and structure. However, microbes remain highly mutable, even when fully "mature." This metabolic plasticity can make it difficult to determine where physiological adaptation to the immediate surroundings ends and true development to a final, programmed state begins. As a result, microbial community development cannot always be directly equated with eukaryotic development.

When the plasticity of many species at many stages of development is combined in a microbial community, studying development becomes complicated, and the distinctions between programming and synergistic community development may be irrelevant. However, there are clear examples of traditional developmental programming influencing single species communities. Fruiting body formation among the myxobacteria is an excellent example. Initiation of fruiting body formation occurs under nutrient limitation and results in development of complex, programmed multicellular structures that contain differentiated, stress resistant spores. This process is mediated, at least in part, via exchange of a series of diffusible signals and cell-contact dependent recognition that drive the direction and rate of development.

Among multispecies communities the clear examples are not numerous, but they do exist. The "genus" *Chlorochromatium* is actually a paired community that acts as a single organism. One partner is a phototroph that can respond to light, and the other member is a heterotroph that provides motility for swimming to that light. Clearly, formation of this two-member community is developmentally programmed, since the member species are inherently designated to achieve this relationship successfully, and they develop to fulfill it. There are other places where one might look for these interactions, including oral communities, bioreactor flocs, and termite guts. There is evidence that other microbial communities form synergistically, relying on multiple interactions between individuals to dictate the eventual architecture of the community.

Another issue of community development and programming is reversibility. Development of microbial communities is much more plastic than classic metazoan development. Microbial development cannot be confined to those processes that are irreversible. However, there are examples of effectively irreversible microbial development. For example, among certain filamentous cyanobacteria, every tenth cell evolves into a heterocyst that fixes nitrogen, and loses the ability to dedifferentiate, or revert to a normal cell structure. Such examples are limited, and most microbial systems retain the ability to revert to autonomous units under certain environmental conditions.

INTEGRATION OF SIGNALS DURING DEVELOPMENT

Difficulties also arise when considering whether microbial communities integrate signals to build communities. Integration, the merging of multiple signals into a smaller set of coherent signals that drive organization of a community, probably does not play a role in mixed-species microbial community development, as there is no central processing function within communities. "Integration" at the community level is not necessarily required for ordered community development. Rather, the responses of individual cells to their environment, including their neighbors, is combinatorial within the community, and manifests itself as the overall phenotype of that community. In this way, each organism responds or integrates independently and it is not necessary to postulate integration across species.

How microbes in communities react to signals will be illuminated by further studies of phenomenon like quorum sensing, a type of signal production governing gene expression. Quorum sensing has been observed in more than 30 species of gram-negative bacteria and a number of gram-positive species. Having been found in pure culture, as well as in intact microbial communities, quorum sensing runs the gamut of microbial settings. In quorum sensing, the chemical signal, an N-acyl-homoserine lactone, for example, can be used as a measure of the population density in a given environment. When density reaches a critical level, the signal accumulates and this increased signal concentration tells the bacteria that they have reached a quorum. In turn, they respond coordinately in some prescribed fashion. For example, quorum sensing in a pathogen may turn on synthesis of factors that are only useful when present in a large amount, such as an antibiotic or a virulence factor. Hence, the gene product of interest is not produced when the microbe first invades the host, but it is "turned on" all at once in response to the appropriate signal. In some cases, it has been shown that this type of signaling is necessary for biofilm development, directly connecting this signaling process to community development.

MICROBES RESPOND TO THE PRESENCE OF OTHERS

Research has uncovered many examples of microbes responding to the presence of other species. This is a critical characteristic of some communities, which apparently rely on these interactions to develop an appropriate structure or to optimize metabolic processes. The microbes in dental biofilms, for example, show a stepwise aggregation that can only be by interactions among the various members of the community. Bioreactor flocs, the engineered assemblages of microbes that accomplish a given metabolic process, also exhibit interactions that determine the relative positioning of the members.

Another example of interspecies communication is syntrophic hydrogen transfer, which is carried out in mixed communities. Interspecies hydrogen transfer allows microbial communities to carry out process that would be energetically impossible without the contributions of each species. In these cases, one syntrophic partner carries out a process that under normal conditions is energetically unfavorable: the production of hydrogen. The other microbial partner consumes hydrogen, thereby allowing the hydrogen-producer to continue with the otherwise unfavorable process. In these situations, cells of the two species come into close contact with one another, allowing for the efficient transfer of hydrogen, little accumulation of this valuable substance, and minimal loss to diffusion.

Mechanisms for Positioning Cells

Within many kinds of microbial communities, secondary structure can buffer sensitive members from environmental fluxes for sensitive members, maximize the ability of two or more species to carry out coupled functions, and create micro-environments conducive to exotic, localized forms of metabolism. For some communities, this structural organization is apparently governed on a community-wide scale, via mechanisms like quorum sensing, in which a signal from every member of the community contributes to the architecture of the community. In other communities, complex configurations can come about in seemingly uncontrolled ways that are actually closely guided by pair-wise interactions. For example, the communities harbored in termite guts that allow the insects to digest cellulose are highly structured, but it is thought that this organization results from small-scale interactions, rather than from an overall architectural plan.

In order to achieve the appropriate secondary structure for a community, cells must position themselves, moving within the community to the appropriate locations. It is likely that many different mechanisms control this cellular positioning, but most communities apparently employ chemical gradients to dictate the appropriate movements. An example of gradients in action is the migration of cells within microbial mat communities. Microbial mats are characterized by steep gradients of sulfide and oxygen. Sulfide-oxidizing bacteria, like *Beggiatoa*, position themselves along this gradient to optimize their ability to make energy from transferring electrons from sulfide to oxygen. At night, when oxygen is limiting in the mat, *Beggiatoa* glide close to the surface, where atmospheric oxygen is available. During the day, when oxygen is produced by photosynthetic members, oxygen is present deeper in the mat, and *Beggiatoa* glide downwards, where oxygen and sulfide concentrations are optimal.

Scales of distance

The distances between cells in a microbial community can be critical to community functions, and distances ranging from the length of the entire community down to the length of an individual cell can play a role. Many communities require that very short distances be maintained between cells. *Myxococcus*, for example, requires “head-to-head” contact between cells in order to initiate fruiting body development. Likewise, the collaborative interspecies hydrogen transfer between methanogens and ethanol fermenters requires extremely close contact, as do all known communities that carry out interspecies hydrogen transfer.

5.0 Technologies Available for Studying Microbial Communities

There are many techniques available for the study of microbial communities. Conversely, there are also many opportunities to develop new methods. Although numerous biological, chemical and physical techniques may be brought to bear on the study of multicellular microbiology (see list below), two general approaches have revolutionized the study: advanced imaging technology and molecular probing. Advanced imaging techniques apply an analysis, which can be automated, to an image or many images of a microbial community, allowing measurements of any of a number of different parameters, including the genetic and phenotypic diversity of the community, the physical distribution of different members within the community, or the viability and metabolic activity of the community and its members. Molecular probing entails the use of a nucleic acid or immunological component to label and identify the genetic or metabolic contributions of different members of a community.

Microbial communities operate on many different scales, and techniques applied to the study of a particular community should be tailored accordingly. From the thin oral biofilms that form on the surfaces of teeth to the vast, continuous communities of cyanobacteria in the open ocean, microbial communities run the gamut in size and impact, and the analytical methods used in characterizing them should be applied with an eye to the scale in which the investigator is interested. Many tools are available for these analyses, ranging from microscopic techniques to satellite imagery, and researchers need to maintain a focus on the scale of the question at hand when selecting among them.

TABLE 1
ANALYTICAL TECHNIQUES FOR
THE STUDY OF MICROBIAL COMMUNITIES

In situ Techniques and Microscopy/Staining
- Non destructive EM and AFM
- SEM
- CSLM
- StarFISH – combination labeling and FISH
- Viability staining and Metabolic activity

Molecular and Genetic Techniques
- Mutant Screens
- Reporter Genes
- Genomics, Proteomics, Metabalomics
- Antibody Libraries
- 16S-rRNA Gene Techniques
- TRFLP and DGGE
- Q-PCR
- Metagenomics

Spectroscopic techniques
- IR
- MR
- NMR
- FTIR

Microprobes

Physical techniques

Natural isotope techniques

In Situ Techniques for Examining Microbial Communities

Two distinct types of *in situ* techniques are available: nondestructive real-time monitoring and destructive techniques that kill the bacteria while retaining the original structure of the community. To study microscopic biofilm communities, both types of methods are heavily dependent on confocal scanning laser microscopy imaging techniques. In destructive techniques, cells from any microscopic microbial community can be fixed and embedded in resin prior to staining and imaging. In non-destructive techniques, the community must be grown in a flow cell that allows microscopy of the living community from early colonization through to the achievement of a climax community. Among nondestructive *in situ* techniques for studying microbial communities, optical fiber imaging holds particular promise for the field. Advanced fiber optical imaging systems are being developed that can provide a confocal image, allowing a three-dimensional view of the community. Advances are also being made in the resolution and depth that can be achieved by the technique.

Both nondestructive real-time monitoring and destructive techniques are critically important to the study of microbial communities. However, there is a tendency for these approaches to be descriptive or qualitative and poorly controlled. Efforts are being made to develop more rigorous techniques that allow quantitative measurements and appropriate controls while retaining the structure and viability of the community under study. It must also be kept in mind that *in situ* techniques like these are a complement to invasive, reductionist approaches, not a replacement for them. Destructive techniques that dissect the community are of equal importance and need to be incorporated into the experimental design.

Molecular Techniques

The use of molecular techniques has radically changed the practice of microbiology research, allowing fine-scale analyses of microbial communities that were never before possible. Within the past 20-30 years, microbiologists have implemented these powerful genetic and biochemical techniques, shedding light on the development of microbial communities, the relationships within communities, and the contributions of individual populations. Some specific issues and cautions associated with the use of individual molecular techniques are discussed below.

REPORTER CONSTRUCTS AND EXPRESSED SEQUENCE TAGS

Reporter constructs, particularly those coupled with fluorescence microscopy, play an important role in studying microbial communities. A reporter is an exogenous gene that has been placed within the genome of a microbe to signal the activity of another, endogenous gene of interest. The reporter gene is transcribed and translated into a protein when the gene of interest is activated by the host, and it demonstrates a visible or otherwise easily measurable signal. In this way, the expression and activity of a gene that belongs to the host can be determined by measuring these parameters in the reporter protein. For example, the green fluorescent protein (GFP) reporter can be placed in the vicinity of the promoter of a gene that is expressed in the presence of a heavy metal. Under the microscope, cells that produce the protein will fluoresce, highlighting those areas where the heavy metal is present. New reporters with more specificity and applicability to a wider range of target functions are needed in the field.

Another tool in the arsenal of molecular techniques is the molecular probe, a DNA or RNA sequence fused with a fluorescent molecule that is complementary to the mRNA sequence of a protein of interest. If the gene is transcribed by a member of the community, the molecular probe adheres to the mRNA and fluoresces, identifying the location and relative level of transcription. Reporters and tags allow measurements of gene expression and activity under given conditions, providing information about the activity and functions of the member of interest.

GENOMICS AND PROTEOMICS

Genomics is a rapidly evolving field, and the definition of the term “genomics” is debatable. For the purposes of microbial community analysis, genomics may be defined as an array of analytical techniques that exploit the genome, that is, the entire genetic complement of an organism, to determine some attribute of that organism. In other words, genomic techniques utilize the entire genome or genome fragments of an organism to determine the activity or role that organism plays in the community. Generally speaking, genomic techniques are separated from other genetic techniques by focusing on aspects of part or all of the genome, rather than aspects of individual genes.

Although it is a relatively new window on the microbial world, genomics is already used to great advantage in studying microbial communities. DNA microarrays are often used to evaluate gene expression in individual species or mixed communities. A microarray is a device that has been spotted with DNA representing the genes from the microbe or community of interest. In the lab, the microbe is cultivated under any of a number of different conditions, for example, in the presence of oxygen or in the absence of oxygen. The mRNA is extracted from these cultures and added to the microarray device to determine which genes are “turned on” or “turned off” under different conditions. The locations on the array that give off a binding signal indicate which genes were expressed in the culture. This enables the researcher to identify those genes that are necessary for survival under particular conditions or to identify the genes that merit further study.

Though interesting information may be derived from whole genome analysis of almost any microbial community, it is recommended that the communities chosen for these efforts should be carefully selected so that they are highly representative and have the potential to inform other areas of microbiology and ecology. It is also recommended that genomics be applied at very small scales in order to assess heterogeneity within communities. Oftentimes, the scale of experimental design is inappropriately large, which may lead to overgeneralizations about microscale community interactions and processes.

In some cases, the genome of a microbial community can be treated as a single entity, and work is currently underway to sequence the genomes of entire communities. These metagenomic approaches, which treat the genetic content of communities as one large sample of genes and DNA (or Environmental-DNA, e-DNA), are permitting an understanding of the genetic potential within microbial communities without having to know detailed information regarding the individual members of that community.

Proteomic techniques focus interest on proteins, using the entire complement of proteins produced or on a subset of the proteome to determine the activity or contribution of an organism to the community. Proteomics could potentially have an important place in microbial community analysis, but the technical issues involved in identifying proteins from multiple microbes within a mixed population remain daunting.

The development of new "-omics," techniques that take a step back from the reductionist approaches of traditional molecular techniques to analyze parameters that are a function of whole-organism or community-wide characteristics, may be on the horizon. Metabalomics, for example, is already an accepted suite of techniques. The comprehensive study of the sugars and polysaccharides that make up exterior of cells—e.g., glycomics—is a particularly important area to develop in the study of microbial communities, which often rely on these substances for community adherence and structure. Community analysis is wide open to such large-scale analysis techniques, but, again, the scale of sampling must always be carefully considered.

MICROSAMPLING

Microsampling, which can be used to extract samples as small as a single cell from a community, is a powerful way to analyze activities within communities. Laser capture microscopy, optical tweezers, and advanced cell sorting approaches allow access to single cells or small patches of cells, enabling the analysis of environments, gradients, and physiology on microscopic scales. Further development of these techniques will greatly advance our ability to dissect the activities and composition of biofilms over very limited spatial scales.

INTEGRATING DIFFERENT TYPES OF MEASUREMENTS

In order to obtain a comprehensive picture of microbial communities, there is a need to integrate multiple levels of analysis. It has been shown repeatedly that the most powerful studies are those that combine many technologies to tackle a single question. For example, studies that sequence and analyze community ribosomal RNA and investigate the physiology of individual species provide a simultaneous view of community diversity and activity. The use of multiple technologies in microbial community analysis is critical to gaining an accurate, integrated view of the many facets these assemblages present.

AVERAGING DATA FROM COMMUNITIES

Analytical techniques that provide an average picture of a microbial community are essential for establishing baseline activity, but they may mask significant micro-heterogeneity. In the analysis of microbial communities, it is critical to integrate the results of studies on multiple scales of resolution to derive meaningful conclusions. For example, some community-wide measurements would identify the pH in a dental plaque community as being fairly constant, but studies of micro-heterogeneity have shown that areas of extremely low pH (which may be the initiation sites of dental caries) exist. Therefore, techniques that average measurements over an entire community should be complemented with approaches that investigate gradients of gene expression and activity over very short distances. In some situations, the various measurements of community activity, gradients, species distribution, and other parameters may cancel out over the scale of the entire community, but the fine details are nonetheless important to a thorough understanding of community dynamics.

Technologies Needed to Advance the Field

There are many areas of microbial communities research that require development of new analytical methods. For example, new staining and imaging approaches, coupled with improved confocal microscopes that allow more rapid data collection, would provide clearer views of the physical structures of microbial communities. The lack of methods for analysis of cell surface structures, such as matrix material within biofilms, is severely limiting for the field and presents an area that should be developed. Moreover, tools for rapid diversity analyses, like microarrays that allow assessments of phylogenetic composition within communities, are needed.

Improvements of existing analytical methods are also necessary. For example, enhancement of noninvasive, microscale sampling and physical measurements should be explored. In research of microbial biofilms, the shortage of available technologies often limits the rate of advancement in the field, and more focus on methods and technology development for this area in particular is warranted.

TABLE 2
WIDER APPLICATION OF TECHNOLOGIES TO BENEFIT MICROBIAL COMMUNITIES RESEARCH

- Single Cell Technologies
- Additional Staining \ Imaging approaches
- Noninvasive Microscale Physical Measurement (NMR)
- Rapid Detection of Initial Colonization – Blood Chemistry – Probes
- Tools to Examine Cell Surfaces – Carbohydrate Chemistry/ Structure Within Communities
- Rapid Diversity Analysis
- Microflow Cell Tools
- Confocal Laser Microscopy: *in situ*, multiphoton, better image analysis capabilities
- Atomic Force Microscopes
- Non-destructive Electron Microscopy
- Better Image Analysis to Quantify and Characterize Community Structure
- Novel Microbial Cultivation Techniques

6.0 Outstanding Knowledge Gaps

The current understanding of microbial communities, their structure, development, composition, and diversity, is at best incomplete and imperfect. Recognized knowledge gaps include the contributions of organisms that cannot be studied in culture, the mechanisms behind enhanced antimicrobial resistance in communities, and the significance of contact-dependent gene regulation among the diversity of microbial communities. In addition, it should be noted that relevant model systems for addressing these knowledge gaps are few. Model systems should be developed further in order to enable researchers to extrapolate a thorough understanding of a limited number of systems to the larger diversity of microbial communities. Microbes play crucial roles in human health and in ecological and industrial processes. Improving our understanding of the known knowledge deficiencies will not only enlighten the management and use of communities in these roles, but will also reveal new arenas for research and implementation of microbial communities.

What are the Contributions of Not Yet Cultivated Microorganisms?

The issue of uncultivated organisms rings loudly in the ears of all microbial ecologists, particularly those concerned with the interactions of cells in communities. It has been estimated that as many as 99% of all microbial species have not yet been grown in culture, and many may, in fact, be resistant to cultivation by available techniques. As a result, there are limited opportunities for studying the genetic, biochemical, and metabolic capacities of the vast majority of single-celled organisms. This discrepancy—that most of the microbes in the world cannot be studied in fine detail in the lab—is at the root of what may be an immense gap in our understanding of the microbial world.

Oral biofilms offer a good demonstration of this gap in the current knowledge. When viewed with a microscope, it is clear that half of the organisms in dental plaque are spirochetes, with distinct spiral-shaped morphology and corkscrew motility, but most of the identified cell types have not yet been grown in the lab. Many such uncultivated organisms are apparently active in the community, and they likely play a role in oral health. In many communities, including the oral flora, it is not known whether active but uncultivated microbes contribute a large extent or a small percentage to community activity, and it remains a source of active debate and investigation.

A number of explanations have been proposed for the apparent unculturability of many microbial species. First, it is highly likely that many microbes are, in fact, culturable through the use of current technology, but they resist cultivation because the precise growth conditions have not been made available. Some recent successes bear this theory out. In the case of *Campylobacter*, it was only lately understood that microaerophilic conditions are needed for its growth. A recent study evaluated the percentage of clinical vaginal swabs from which *Staphylococcus* aureus can be grown, and showed that 90% of swabs were reported back as negative for this species. However, when growth plates were incubated for a longer period than that originally dictated by the clinical protocol, the organism was found to have developed from many previously “negative” samples; this almost universal presence of this potential pathogen was confirmed in a separate study by the use of FISH probes and PCR. In this case, the culture conditions were defined by clinicians’ need to get fast results, but they limited the validity of the final result.

Another cause for poor culturability may be that critical elements present within the microbial community environment are lacking in traditional cultivation techniques. Current cultivation techniques will need improvement if investigators hope to capture the organisms that require the nutritional, chemical, or physical support of the surrounding microbial community.

It is important to distinguish between metabolically active microbes that cannot be cultivated due to our limited knowledge of their physical and nutritional requirements and those organisms that are quiescent or dormant and cannot be revived in culture. Clearly, microbes in the first category are expected to have enormous impacts on the activity of their respective microbial communities. Microbes that fall in the second category may also impact the community, as they may contribute to the structure of biofilms, for example, but do not deplete resources. Dead cells may, in fact, release nutrients into the community that support the growth of other members.

More research is needed to investigate the contribution of non-cultivatable cells within microbial communities. Fortunately, we now have some tools that allow for this type of research. The application of meta-genomics approaches, in which DNA is extracted from a total sample without separating out the individual organisms and the resulting library of genetic sequences is analyzed, is proving fruitful, and driving new questions for research. For example, research has shown that a metagenomic library of soil microorganisms that is probed for 16S ribosomal RNA (rRNA) sequences may contain up to 25-30% *Acidobacter* sequences. Only three members of this genus have been cultured, leaving investigators with many questions (and few answers) regarding the metabolic role of these organisms in soil. An important caveat when interpreting non-cultivation based discoveries like this is the all too frequent disconnect between genetic fingerprints and metabolic capabilities. The genetic sequences of marker loci like the 16S rRNA gene are only hints about the phenotypic characteristics of a given organism. Nevertheless, metagenomics and other molecular techniques will allow investigators to make major inroads into the world of viable, but not yet cultivated, microbes.

For many years, microbiologists relied solely on traditional culture-based approaches, in which only those microorganisms that could be grown in a lab were used to extrapolate to the whole of microbial diversity and microbial communities. Over-reliance on these techniques has likely introduced a number of misunderstandings with regard to the *in situ* activity of microorganisms. The use of cultivation-based techniques in modern-day research should be examined carefully, and extraneous experimentation that would lead only to conclusions relevant to the bench-top behavior of pure-culture microorganisms should be limited. The focus of current and future research should be on the wider world of microbial diversity, and should not be limited to those few non-representative organisms that are easily maintained in the laboratory environment.

Enhanced Resistance to Antimicrobials in Microbial Communities

Biofilm microbial communities often exhibit heightened resistance to antimicrobial treatment as compared with their free-living counterparts. Antimicrobial resistance in biofilms and other microbial assemblages is a critically important issue and has extensive practical implications for medicine, industry, and the environment. It is widely agreed that the field merits a great deal more experimental work.

Who is a member of the community and who isn't?

One point that is so fundamental to the topic of microbial communities that is often overlooked is the question of how to define the limits of a microbial community. In the environment, functionally defined microbial communities exist in continuity with one another, and the distinctions between them blur. It is difficult to delimit these communities in almost any habitat, separating the cells and species that are members from those that are not. Resolving this issue should be a key theme for discussions among investigators, and an attempt should be made to come to some agreement on what defines a member of a given microbial community.

What are the right model systems for community interactions?

A major missing component in microbial community research is the development of one or a few good model systems to determine the fundamental mechanisms at work in communities. An ideal model system would be comprised of well-characterized individual members that together accomplish functions that are beyond the capabilities of the individual species. While the study of this model system should not preclude investigating the diversity of microbial communities, some significant effort should be directed towards developing one or more systems. Initially, work should focus on characterizing communities with only two member species. *Chlorochromatium* is an excellent candidate for such a model. Once inroads have been made into understanding two-member communities, further research can branch into investigating multi-species communities. Multispecies candidates include dental communities, microbial mats, termite or gypsy moth gut communities, and the communities that inhabit the rumens of cows.

7.0 Education, Training, and Collaboration

The Role of Multidisciplinary Collaboration

Microbial communities are complex and phenomena related to their development, function, dynamics, and impacts are not limited to the domain of any single traditional scientific discipline. Hence, research into microbial communities requires the intellectual and technical skills of professionals from many different fields, including microbiology, ecology, medicine, population biology, environmental chemistry, molecular biology, biochemistry, soil science, plant science, hydrology, geology, engineering, and others. Because of the breadth of skills required in this field, the development of multidisciplinary collaborations is strongly recommended.

Table 3 identifies pairs of traditional disciplines that are particularly well suited to collaborative efforts and areas of research that are best suited to these types of investigations. Interest in collaboration depends to a large extent on attracting diverse researchers to this arena and may be accomplished through seminar or symposium sponsorship at large scientific meetings, such as the meetings of the American Society for Microbiology (ASM). Recruitment may also be accomplished by individual scientists, who, already engaged in research on microbial communities, recognize persons outside the field who may contribute and propose collaborations. However, successful collaborations can only be established if financial resources are available to support them. Scientists need to encourage funding agencies and corporations to both recognize the need for multidisciplinary work and to encourage joint projects by funding specific grants and research.

TABLE 3
COLLABORATIVE OPPORTUNITIES IN
MICROBIAL COMMUNITIES RESEARCH

Potentially useful interdisciplinary collaborations (some of which are already being funded to some extent) include:

- modeling and experimental research programs
- environmental scientists and clinical scientists
- ecologists and molecular biologists
- evolutionary biologists and microbiologists

Research areas that are particularly appropriate for collaborative work include:

- context of structured communities
- drinking water biofilms
- correlating the introduction of bioterrorism agents with microbial communities
- remediation following a bioterrorism incident
- prevention and reduction of bioterrorism agents
- beneficial applications of biofilms

Education and Training

In addition to multidisciplinary research efforts, the complexity of microbial communities necessitates multidisciplinary education and training. In the future, more students at the undergraduate and graduate levels should be exposed to the “communities view” of microbial biology. Moreover, students should receive integrated training in order to develop experience in multiple relevant disciplines.

Small meetings, such as ASM and Gordon conferences, have advanced the field of microbial communities research and education and their continued support is encouraged. Furthermore, short courses for graduate students and early faculty that incorporate the study of microbial communities have proven useful.

International Collaboration

International collaboration between scientists active in the field of microbial communities research is highly desirable. Scientific research should have no borders, and international collaborations should be fostered, particularly in the study of microbial communities, which holds great significance the vitality of the planet and all the world’s citizens.

There is a need for more direct mechanisms of exchange among international laboratories, such as exchange programs for graduate students and postdoctoral fellows and increased support for visiting international professorships. European efforts of this type, which include money for travel and meetings among collaborating scientists, could serve as examples. Institutions that might sponsor such international collaborations include the Fogarty Center at the National Institutes of Health, the World Health Organization (WHO), and the Gates Foundation. Biofilm workshops throughout the world sponsored by the National Science Foundation have been successful in advancing international research on the topic, and more ventures like them should be encouraged.

Communicating the Importance and Practical Benefits of Research on Microbial Communities to the Public

Scientists need to communicate to the public the crucial role of microbial communities in everyday life. From the major biogeochemical cycles to medical implications to the possibilities for new commercial applications, microbial communities impact our lives in innumerable ways, and a greater awareness of this fact among the lay public would foster a greater ability to enhance the well-being of humans and the environment.

Importantly, these functions and potential applications are accomplished by microbes about which we know little, that have never been cultivated, and that operate in nature not as single species or individuals of a species, but in aggregates or communities. With greater recognition of the importance of microbial communities will come greater support in the public sector for the research that fills these knowledge gaps and improves the human condition.

In certain areas, improved understanding of microbial communities can directly benefit the public. For example, in the medical profession, disease is now recognized as a perturbation of the natural state, and microbial communities frequently play a role in maintaining that state. A grasp of this role can guide patients and consumers in preventing harmful perturbations in their own bodies, directed by the insight that maintaining a healthy flora is more attractive than medicines to amend perturbations.

In addition to receiving information about benefits to health and the environment, the public should be made aware that microbial communities comprise a vast, untapped commercial resource. The potential applications of microbial community functions are numerous and are limited only by the imaginations of scientists and engineers. Possible uses of microbial communities include large-scale applications for pollution reduction and water and sludge treatment. One can envision a next technological revolution after the electronic software revolution—a "bioware" revolution with microbial community products effectively reducing biofouling and corrosion, drug discovery, and application of mixed communities in industrial fermentation processes. Microbial processes can be exploited to their full potential if scientists can identify the basic mechanisms of community establishment, function, and maintenance.

Improved public communication of current understanding of microbial communities can be carried out by non-academic science advisory groups, academic researchers, and government agencies. Preparing and disseminating documents designed for public information purposes, such as this colloquium report, are crucial to this effort. The Internet is a particularly powerful tool for disseminating information. Individual researchers could make information available to an extremely large audience by posting websites on their own work or on the general topic of communities. These websites should be written in lay language, with visual aids and interactive elements.

Educational materials, like television programs and articles in popular magazines, can also be used to educate the public. When targeting young audiences, microbiology education needs to incorporate up-to-date subject matter regarding microbial communities so that young students will be familiar with these ideas as they are trained. In school, science laboratory exercises should include investigations of microbial communities.

Scientific societies are perfectly positioned to support education and communication activities, and they should be encouraged to continue to hold meetings on microbial communities. Both symposia at national scientific meetings, which communicate to fellow scientists, and meetings targeted to the public, which can attract journalists from the popular media, are helpful in educating a wide audience.

8.0 Summary of Recommendations

Recommendations for Research

- Determining the contributions of organisms that cannot be cultured in the lab to the development, structure, and function of microbial communities should be an overriding theme for future research in this field.

- Understanding the reasons for enhanced antibiotic resistance of microbial communities is pivotal to managing persistent microbial community infections.

- Development of suitable model systems for the study of microbial communities would prove profitable to the field by enabling a thorough understanding of the underlying order and processes at work in these complex and dynamic systems.

Recommendations for Education and Collaboration

- The phenomena relevant to microbial communities research are not the exclusive realm of any single scientific discipline. Multidisciplinary collaboration among scientists from many fields is most conducive to making important contributions in the areas where the current knowledge is weakest.

- International collaborations are critical if the strengths of research programs in the various nations involved in the field are to be used to their full potential.

- The public is largely unaware of the innumerable impacts that microbial communities have on daily life. Improving the education of the public through the use of publications, television, and the Internet is encouraged so that the public can come to recognize the importance of continued research in the field.