"BioPhysics Research"

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An organic, biodegradeable microbial growth enhancement agent

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MetaGenomics

Why *Meta*Genomics?

Microbes run the world. It's that simple. Although we can't usually see them, microbes are essential for every part of human life—indeed all life on Earth. Every process in the biosphere is touched by the seemingly endless capacity of microbes to transform the world around them. The chemical cycles that convert the key elements of life—carbon, nitrogen, oxygen, and sulfur—into biologically accessible forms are largely directed by and dependent on microbes.

All plants and animals have closely associated microbial communities that make necessary nutrients, metals, and vitamins available to their hosts. Through fermentation and other natural processes, microbes create or add value to many foods that are staples of the human diet. We depend on microbes to remediate toxins in the environment—both the ones that are produced naturally and the ones that are the byproducts of human activities, such as oil and chemical spills. The microbes associated with the human body in the intestine and mouth enable us to extract energy from food that we could not digest without them and protect us against disease-causing agents.

These functions are conducted within complex communities—intricate, balanced, and integrated entities that adapt swiftly and flexibly to environmental change. But historically, the study of microbes has focused on single species in pure culture, so understanding of these complex communities lags behind understanding of their individual members. We know enough, however, to confirm that microbes, as communities, are key players in maintaining environmental stability.

By making microbes visible, the invention of microscopes in the late 18th century made us aware of their existence. The development of laboratory cultivation methods in the middle 1800s taught us how a few microbes make their livings as individuals, and the molecular biology and genomics revolutions of the last half of the 20th century united this physiological knowledge with a thorough understanding of its underlying genetic basis. Thus, almost all knowledge about microbes is largely "laboratory knowledge," attained in the unusual and unnatural circumstances of growing them optimally in artificial media in pure culture without ecological context.

The science of metagenomics, only a few years old, will make it possible to investigate microbes in their natural environments, the complex communities in which they normally live. It will bring about a transformation in biology, medicine, ecology, and biotechnology that may be as profound as that initiated by the invention of the microscope.

What is METAGENOMICS ?

Like genomics itself, metagenomics is both a set of *research techniques*, comprising many related approaches and methods, and a *research field*. In Greek, *meta* means "transcendent." In its approaches and methods, metagenomics circumvents the unculturability and genomic diversity of most microbes, the biggest roadblocks to advances in clinical and environmental microbiology. *Meta* in the first context recognizes the need to develop computational methods that maximize understanding of the genetic composition and activities of communities so complex that they can only be sampled, never completely characterized. In the second sense, that of a research field, *meta* means that this new science seeks to understand biology at the aggregate level, transcending the individual organism to focus on the genes in the community and how genes might influence each other's activities in serving collective functions.

Individual organisms remain the units of community activities, of course, and we anticipate that metagenomics will complement and stimulate research on individuals and their genomes. In the next decades, we expect that the top-down approach of metagenomics, the bottom-up approach of classical microbiology, and organism-level genomics will merge. We will understand communities, and the collection of communities that forms the biosphere, as a nested system of systems of which humans are a part and on which human survival depends. In some situations, it will be possible to apply the new understanding to problems of urgency and importance. Metagenomics in either sense will probably never be circumscribed tightly by a definition, and it would be undesirable to attempt to so limit it now, but the term includes cultivation-independent genome-level characterization of communities or their members, high-throughput gene-level studies of communities with methods borrowed from genomics, and other "omics" studies, which are aimed at understanding transorganismal behaviors and the biosphere at the genomic level. Although in its current early implementation metagenomics focuses on non-eukaryotic microbes, there is no doubt that its concepts and methods will ultimately transform all biology.

In just this way has genomics, a science developed to aid the advancement of biomedicine and the understanding of our own species, transformed the science of all organisms and the application of that science in epidemiology, clinical microbiology, virology**, agriculture, forestry, fisheries, biotechnology**, microbial forensics, and many other fields.

What Microbes Can Do

There are countless ways in which microbes influence daily life. Earth is a biological entity as much as it is a physical one, and most of the vital biology, on which all life depends, is *micro*biology. But because microbes are individually invisible, we (even microbiologists) need to be reminded of our debt to them.

Microbes Modulate and Maintain the Atmosphere

Carbon is the most abundant chemical element in all living things, including humans (excluding the hydrogen and oxygen in the water, which makes up the bulk of our weight). Carbon dioxide (CO_2) in the atmosphere is the most abundant source of carbon on Earth, but in this form it is inaccessible to animals and most bacteria. Plants and some bacteria "fix" carbon through photosynthesis, a light-driven conversion of CO_2 to sugars that generates the oxygen that fuels all aerobic forms of life. Although plants tend to get most of the credit, bacteria are responsible for about half of the photosynthesis on Earth.

Microbes Keep Us Healthy

It should come as no surprise that in the microbe-dominated biosphere, close relationships between microbes and animals are an ancient theme. Humans are no exception. The numbers are staggering. The microbes that reside on the surface of the human body alone outnumber human cells by about a factor of 10. The genomes of members of our indigenous microbial communities (the human metagenome) contain thousands of times more genes than the human genome.

Understanding how microbial community structure affects health and disease may contribute to better diagnosis, prevention, and treatment of disease. potentially.
In light of the crisis in management of infectious pathogens due to emergence of antibiotic resistance, we would be well served to understand the role of microbial communities in protecting from infectious agents.

Microbes Support Plant Growth and Suppress Plant Disease

The microbial communities on and around plants play a central role in the health and productivity of crops. The most complex of these communities reside in the soil, which is a composite of mineral and organic materials teeming with bacteria and archaea. Some functions of these microbes are well known. Some bacteria fix atmospheric nitrogen, converting it from dinitrogen gas—a form unusable by plants and animals—to ammonia, which is readily used. Other soil microbes recycle nutrients from decaying plants and animals, and others convert elements, such as iron and manganese, to forms that can be used for plant nutrition. Soil microbial communities determine whether plants will become infected by pathogens. A lingering mystery is the "suppressive soil" phenomenon. In some soils, plants stay healthy even when pathogens are present at high density; when the soil is sterilized, the disease suppression disappears, suggesting a biological basis of the phenomenon. However, in only very few cases has a single microbe isolated from a soil been able to duplicate the suppression. After decades of wrestling with the enigma of suppressive soils, plant pathologists have concluded that in many cases a complex community is responsible for the suppressive activity, which is hugely beneficial to agriculture. No organism has been found to provide the same effect in isolation, because the community members modify each other's behavior.

Microbes Clean Up Fuel Leaks

There are hundreds of thousands of underground storage tanks in the United States, most of which are used for storing gasoline. In fact, almost every corner gasoline station in the United States uses three or more of these tanks to dispense regular, premium, and super-premium versions of gasoline. The sad truth about these underground tanks is that the vast majority of them are already leaking or will leak and send gasoline into the subsurface, where it has the potential to contaminate the groundwater. Given the ubiquity and magnitude of the gasoline leaks and the fact that 50% of the U.S. population relies on groundwater as a drinking-water source, one must wonder how it is that we are not all drinking water contaminated with gasoline!

The answer is that we are being protected by the omnipresent and vastly adaptable subsurface microbial community. As gasoline is released into the subsurface, relatively dormant members of the microbial community are triggered to become active and biodegrade the gasoline constituents. Gasoline is composed of thousands of organic chemicals and a variety of microbes containing complementary metabolic systems are required to degrade them all. Furthermore, because there is too little of any single electron acceptor in the subsurface to react with all the electron donors of gasoline, different bacteria with different respiratory capabilities are required to complete the gasoline remediation. For example, when oxygen is depleted in the groundwater in the vicinity of a gasoline spill, bacteria that can respire nitrate take over, followed by bacteria that respire iron, manganese, sulfate, and, eventually, CO_2. This complicated community of microbes works together in a self-organized pattern triggered by the movement of the leaking gasoline until the contaminants have been transformed into harmless CO_2 and water. The microbial community then becomes dormant again, awaiting the next influx of substrate (either natural or anthropogenic) to return to activity.

Invisible Communities: Global Impact

Modulating the atmosphere, keeping humans and plants healthy, and cleaning up leaking gasoline are just a few examples of the many things that microbial communities can do. The combined activities of microbial communities shape the face of the biosphere on a global scale. The power of these communities lies hidden in the metabolic versatility of their component species that, acting together, regulate the vast majority of matter and energy transformations on Earth. In a loose analogy, the entire biosphere can be imagined as a sort of "superorganism."

Microbes can "eat" rocks, "breathe" metals, transform the inorganic to the organic, and crack the toughest of chemical compounds. They achieve these amazing feats in a

sort of microbial "bucket brigade"—each microbe performs its own task, and its end product becomes the starting fuel for its neighbor. Virtually all elemental cycles—including the generation, consumption and flux of greenhouse gases or, as noted above, the remediation of spilled gasoline—involve microbial collaborations that are tightly regulated and coupled through microbial community interactions.
Not only can many individual systems—for example, the human gut or such processes as the bioremediation of toxic hydrocarbons—be seen to be the tasks of complex and dynamic microbial communities, but these communities are themselves constituents of even larger systems, predominantly microbial, that collectively make up the biggest and most complex functioning system we know: the biosphere.

For example, until the early 20th century, all nitrogen entering the biosphere was produced from atmospheric nitrogen by microbes, providing the organic nitrogen required for new plant growth.

Understanding Microbial Communities

Given that the microbial collective profoundly influences geochemical and greenhouse-gas cycles, as well as climate and environmental change, it is relevant to ask how well we understand microbial communities. In the past, it was difficult to study microbes in their own environments; microbiologists studied individual species one by one in the laboratory. It now appears that many microbes function in nature as multicellular, often multi-species, entities, sometimes even physically connected (as in biofilms) and often metabolically connected.

One approach that has contributed greatly to understanding all organisms is genomics—learning about the evolution and capabilities of organisms by deciphering the sequence of their DNA. Genomics has also greatly advanced microbiology, but, like pure culture, traditional genomics is limited in its ability to elucidate the dynamics of microbial communities.

Pathogenic bacteria and eukaryotes—such as the causative agents of plague, anthrax, tuberculosis, Lyme disease, candidiasis, malaria, and sleeping sickness have received much attention. But many nonpathogenic archaea and bacteria have also been sequenced, including such beneficial organisms as several species of *Prochlorococcus* and *Synechococcus*, major producers of oxygen in the ocean; *Dehalococcoides ethenogenes*, effective in the bioremediation of soils contaminated with chlorinated hydrocarbons; *Lactobacillus acidophilus*, used in making yogurt; *Bradyrhizobium japonicum*, a nitrogen-fixing symbiont of soybeans; and *Saccharomyces cerevisiae* (baker's yeast).

When attention turned to sequencing the genomes of microbes, the preference for working in pure culture was reinforced. No one knew how difficult it might be to sequence an entire genome, but it was obvious that assembly (using a computer to put the sequenced fragments together in complete genomes) would be vastly more complicated if the pieces belonged to several different organisms.

Until recently, all microbial genome sequences were determined from pure cultures. But in the last few years, more than a dozen microbes that can be physically separated from other major sources of DNA or that greatly predominate where they are found in nature have also been sequenced.

Most Microbes Cannot Be Cultured

In 1985, Staley and Konopka reviewed data on scientists' ability to bring microbes from the environment into laboratory cultivation. The "great plate-count anomaly" they identified was this: the vast majority of microbial cells that can be seen in a microscope and shown to be living with various staining procedures cannot be induced to produce colonies on Petri plates or cultures in test tubes.
It is estimated that only 0.1–1.0% of the living bacteria present in soils can be cultured under standard conditions; the culturable fraction of bacteria from aquatic environments is ten to a thousand times lower still.

Microbial Diversity and Variation Have No Limits

When genetic information from macroscopic organisms (animals or plants) is organized into phylogenetic trees to examine how they are related to one another, one can assume that all the individuals of a given species have virtually identical genomes. For example, the genomes of humans differ from one another by only 0.1%. In contrast, microbial phylotyping coupled with genome sequencing has shown that even if culturability ceased to be a problem, diversity will always be a challenge; indeed, it is a greater challenge than might have been imagined. Hundreds of thousands or even millions would be too low an estimate of the number of genomes that would have to be sequenced in any kind of whole-genome-based metagenomics program. This is due in part to the large numbers of species of microbes in most environments.

Metagenomics Can Contribute to Advances in Many Fields

Metagenomics offers a means of solving practical problems facing humanity. Cracking the secrets of some of Earth's countless microbial communities will reveal ways to meet myriad challenges in biomedicine, agriculture, and environmental stewardship. These are among the most important potential contributions:

- **Earth Sciences:** the development of genome-based microbial ecosystem models to describe and predict global environmental processes, change, and sustainability
- **Life Sciences:** the advancement of new theory and predictive capabilities in community-based microbial biology, ecology, and evolution
- **Biomedical Sciences:** the description, on a global scale, of the role of the human microbiome (the collective genome of our symbionts) in health and disease in individuals and populations, and the development of novel diagnostic and treatment strategies based on this knowledge
- **Bioenergy:** the development of microbial systems and processes for new bioenergy resources that will be more economical and environmentally sustainable and less vulnerable to disruption by world politics

- **Bioremediation:** the development of tools for monitoring environmental damage at all levels (from climate change to leaking gas-storage tanks) and microbe-based (green) methods for restoring healthy ecosystems
- **Biotechnology:** the identification and exploitation of the remarkably versatile and diverse biosynthetic capacities of microbial communities to generate beneficial industrial, food, and health products
- **Agriculture:** the development of more effective and comprehensive methods for early detection of threats to food production (crop and animal diseases) and food safety (monitoring and early detection of dangerous microbial contaminants) and the development of management practices that maximize the beneficial attributes of microbial communities in and around domestic plants and animals
- **Biodefense and Microbial Forensics:** the development of more effective vaccines and therapeutics against potential bioterror agents, the deployment of genomic biosensors to monitor microbial ecosystems for known and potential pathogens, and the ability to precisely identify and characterize microbes that have played a role in war, terrorism, and crime events, thus contributing to discovering the source of the microbes and the party responsible for their use