Microbial Communities in Nature: a Postgenomic Perspective

Understanding microbial communities entails distinguishing active individuals from mere DNA or those cells that are resting or dead

Donald A. Klein

The term microbial community often comes up in the context of natural microbial assemblages (NMAs) being analyzed. Because the majority of microbes in such NMAs show no detectable activity or growth, researchers use molecular techniques to characterize microbes in these NMAs—while assuming that nucleic acids recovered in bulk from such samples represent those microbial communities.

Thus, the word “community,” once used mainly to describe active and interacting organisms, is now also being used in a metagenomic context to describe both active and inactive microbes. This confusion makes it crucial to emphasize that microbial communities consist of active and functioning organisms present in any particular environment. To overcome this confusion and go beyond the limits in analyzing bulk extracted DNA molecules, I recommend conducting molecular analyses on active individual microbes. Such individual microbe-based analyses will also make it possible to consider epigenetic influences on the phenotype of a microbe and to assess intragenomic heterogeneity. This approach thus should provide a more meaningful view of microbial communities, the active microbes that usually are a minor portion of the microbes present in natural assemblages.

Microbial Communities Consist of Active, Interacting Organisms

Ecologists define “community” as “an interacting population of various species in an area.”

Traditional microbial ecologists follow suit. “The ultimate aim of the study of microbial ecology is to understand the interactions within microbial communities and between them and their environments,” notes Madelyn Fletcher, formerly of the University of Maryland and now at the University of South Carolina, and her collaborators in a review. Importantly, interaction and activity are key elements for defining a community in ecological terms.

However, microbial ecologists face a major challenge because so many microbes that are present in natural assemblages—99% is often cited—are inactive or unculturable, and thus should not be considered as a part of the microbial community. Active microbes are those interacting with their im-

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<th>Summary</th>
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<td>• Microbial communities consist of active microbes—typically, the minor portion within soils, waters, and biofilms, or associated with plants and animals.</td>
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<td>• DNA-based analyses of natural microbial assemblages (NMAs) using bulk extraction-based approaches do not provide specific information on the active microbes functioning in particular locations and conditions.</td>
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<td>• Techniques are becoming available to conduct physiological, chemical, molecular, and other analyses on individual, active microbes in situ or after removing them from a particular environment.</td>
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<td>• By using an active cell-based analytic approach, one can analyze epigenetic effects and intragenomic heterogeneity.</td>
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The abiotic envelopes of different microbes, in relation to the abiotic envelope for a NMA, considering temperature and pH variations at a particular site. (a) Microbes can be present in the NMA whose abiotic requirements lie outside of the abiotic envelope for the site. (b and c) Environmentally competent microbes with different and separate abiotic requirements, and microbes (d and e) with overlapping abiotic requirements that can function at the same time. Whether these different microbes become active, and thus part of the microbial community, depends on meeting their specific functional requirements.

mediate abiotic and biotic environment based on physiologically driven, detectable exchanges of resources. Of course, determining which microbes meet this definition depends directly on the detection methods being used. Thus, the spectrum of activity ranges from nongrowing microbes, whose internal reserves are being used for maintenance, to microbes that are actively reproducing and growing. It is critical to measure such activity in situ; detecting it in silico after enrichment is not germane.

The movement of microbes into environments where they cannot function occurs at different rates and on scales ranging from micro-environmental to global. "The question is not

how many numbers and types of fungi can be found in the soil, but what organisms lead an active life in the soil," noted Selman Waksman more than 90 years ago. Further, some species might be found at a particular site merely because they were accidentally transported there, but are not capable of functioning. Similarly, John H. Warcup, of the Waite Institute, Adelaide, South Australia, emphasized the importance of distinguishing between "mere occurrence" versus activity of fungi in wheat-field soil. He, too, noted that some fungal spores might be "from the air."

Some environments might be exempt from these considerations. For instance, deep terrestrial environments such as a mine might contain water that remained undisturbed for tens of millions of years. However, other than such restricted environments, microbes or their nucleic acids appear capable of entering environments where they do not function.

**Seeking Microbial Communities when Studying Natural Microbial Assemblages**

Most microbes in a NMA do not express—and may not be capable of expressing—activity. This inactivity may be due to internal or external factors. To be scored as active, a particular microbe must be environmentally competent and also must exhibit activity in situ (Fig. 1). At least six resource categories (Table 1) are of concern in characterizing natural microbial assemblages, particularly with molecular techniques:

- **Category 1:** environmentally incompetent vegetative cells. A large portion of "unculturable" microbes in a NMA may never grow or show signs of life. One reason is that they might have been carried to the particular environment, and such "peripatetic" microbes might never find conditions appropriate to grow or function as part of the local microbial community.
- **Categories 2 and 3:** inactive and active environmentally competent vegetative cells. Although environmentally competent vegetative
microbes might be located within a permissive abiotic envelope, whether they express activity could depend on their encountering specific nutritional, energetic, symbiotic, or comparable requirements. For example, category 2, or inactive environmentally competent vegetative cells, encounter conditions that are inappropriate for activity when the NMA is being tested, but might test positive when conditions change. Meanwhile, category 3, or active competent vegetative cells, can function in a permissive abiotic habitat in which their nutritional, energetic, or symbiotic requirements are met. These active and functioning category 3 microbes are the microbial community that is a minor portion of microbes in the NMA.

- Category 4: resting cellular entities. A wide variety of resting entities could be present, including endospores (Bacillus), cysts (Azotobacter), heterocysts and akinites (cyanobacteria), and asexual propagules (actinomycetes). Similarly, eukaryotes form resting structures that include sexual and asexual fungal spores as well as protozoan cysts. These or other resting cellular entities may be transported to particular environments.

- Category 5: acellular entities. Viruses and prions are NMA components. In marine systems, for instance, virus titers typically are 10-fold higher than those of bacteria. Moreover, viruses play roles in transferring genes horizontally, controlling host populations, and acting as reservoirs for biochemical capabilities for host organisms, such as Prochlorococcus. Viruses contain nucleic acids that can affect molecular analyses, particularly following bulk extractions.

- Category 6: free nucleic and other cellular components. Nucleic acids, metabolic intermediates, enzymes, and structural components that are released from cells typically are associated with NMA. Of these materials, the nucleic acids are of particular interest, in part because DNA binds to clay minerals and humic substances, reducing its susceptibility to being degraded without eliminating its capacity to transform competent cells, according to Gunther Stotzky and his collaborators at New York University. PCR can amplify free DNA, which also can interfere with single-cell genomic analyses (for instance, for Prochlorococcus). Further, free DNA may come from an organism “not originally growing in the examined habitat,” notes Howard Gest of Indiana University. Simply finding a molecular sequence in a particular environment does not mean that it is associated with an organism that is active in situ. Thus, molecular approaches can be compromised when based on bulk samples of extracted nucleic acids. Moreover, a potentially significant portion of those nucleic acids may have nothing to do with any type of microbe in such natural microbial assemblages.

**Active Individual Microbes, the Microbial Community, and Molecular Techniques**

Separating the active, interacting individual organisms from the bulk of microbes in natural microbial assemblages for analysis can depend on cell sorting or more specific approaches. For instance, to recover individual microbes from complex soil matrices, there are several isolation techniques, including physical micromanipulation, optical tweezers, and laser catapulting. In addition, single-cell PCR and multiple displacement amplification (MDA), which amplifies genomes of individual cells, are becoming routine.

For example, Roger Lasken, now of the J. Craig Venter Institute in San Diego, Calif., and his coworkers conducted genomic analyses of individual bacteria. Similarly, Kun Zhang of the University of California, San Diego, George Church of Harvard University Medical School,

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**Major biotic resources in a natural microbial assemblage**

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<th>Biotic Resource</th>
<th>Part of the microbial community</th>
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<tr>
<td>Environmentally Incompetent Vegetative Cells</td>
<td>No</td>
</tr>
<tr>
<td>Environmentally Competent Vegetative Cells—Inactive</td>
<td>No</td>
</tr>
<tr>
<td>Environmentally Competent Vegetative Cells—Active</td>
<td>Yes</td>
</tr>
<tr>
<td>Inactive Resting Cellular structures—Environmentally Competent/Incompetent</td>
<td>No</td>
</tr>
<tr>
<td>Acellular entities – non-host cell associated</td>
<td>No</td>
</tr>
<tr>
<td>Free Nucleic Acids</td>
<td>No</td>
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*Other soluble and particulate cellular and acellular-derived constituents also are present. Only the category 3, the environmentally competent active vegetative cells, is of concern in terms of describing the microbial community that is functioning under a given set of environmental conditions, particularly when using molecular approaches.*
and their collaborators completed genomic analyses on single cyanobacterial cells. The ability to detect RNA levels and to carry out stable isotope-based analyses also are used for analyzing individual microbial cells.

Working with single cells has limitations. Actively observing a cell may change it, and this issue must be taken into account in designing such experiments. To circumvent this problem, one can successively examine the same cell with fluorimetric and then probe-based methods, followed by growth/activity and other physical, chemical, and molecular analyses.

There are other challenges to face. For example, how can one adequately sample a milliliter of water or a gram of soil via single-cell analyses, when such a sample might contain $10^7-10^8$ observable bacteria as well as other NMA components? Completing this task is largely a mechanical challenge; single-cell analysis based on automation is a rapidly developing field. Thus, mechanizing the process while using large database management methods will facilitate working at this level of resolution. Important advances include the efforts of A. E. Ottesen and Jared Leadbetter of the California Institute of Technology, and their collaborators, to monitor genes in individual cells.

**Defining the Microbial Community in the Postgenomic Era**

Studying individual organisms that are active in situ is a valuable and informative approach for better understanding microbial communities, one with broad implications in this post-genomic era. It is much like entering a forest and determining which individual trees and other plants are active and reproducing, stressed, in resting form as seeds, or dead. We can do the same when analyzing animals, including human populations, in particular locations. However, in following the logic inherent in this analogy, we recognize that people in a cemetery cannot be considered an active part of the human community. Similarly, in analyzing microbial communities, we need to distinguish those individuals that are active and reproducing from those in resting states and those that are dead.

Analyzing microbial communities in this way puts a heavy burden on experimental scientists, who are being asked no longer to rely on one-size-fits-all bulk extractions of nucleic acids to evaluate communities in particular settings. As environmental conditions change in such settings, from aerobic to anaerobic, for example, or when nutrients are added to a particular NMA, different microbial components might become active. Thus, when conditions change, fresh analyses will be needed to determine how the currently active microbial community differs from the one that was active before those conditions changed.

Studying active individual microbes in situ will provide new insights about NMAs and how epigenetics contributes to microbial diversity, phenotype, and intragenomic heterogeneity. To understand such phenomena, we need to know more than DNA sequence information, whether from analyses of 16S or 18S rRNA, rpoB, or genomes.

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**SUGGESTED READING**


