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Commentary

Microbiology in transition

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Mention microorganisms to the layman or even the majority of scientists, and you will evoke images of disease, spoiled food, scum in the toilet tank, etc., but on occasion also nicer images of sourdough rolls cooked by a campfire, good old home brew, or the satisfaction of putting a load of rich compost on the garden. Yet images of this sort reveal more our ignorance than our understanding of the microbial world, for these conventional views amount to little more than the effects, beneficial and otherwise, that microorganisms have on us. Although we see animals and plants in this way too, we also know them in their own right, as it were: We are sensitive to their beauty and grace; we revel in their modes and variety of behaviors; we study their migrations, the details of their social relationships, and so on. This qualified, aesthetically tempered understanding—the roots of which are embedded in our history and culture—does not exist for microorganisms. But for their capacity to foil our best laid plans and inadvertently benefit us, microorganisms would not exist for us. Yet there is a need to understand microorganisms, intimately and well; a need that becomes pressingly evident as the human race tests the balance of the Earth's biosphere, unerringly increasing "The Three Ps"—population, power usage, and pollution. The microbial world established our biosphere and sustains it. As Harvard paleontologist Stephen Jay Gould recently put it (in gentle rebuke of a failure to recognize the fact): ". . . we live in the Age of Bacteria (as it was in the beginning, is now and ever shall be, until the world ends). . ." (1).

Our ignorance, with its threatening consequences, is simply remedied: We (scientists) need only to perceive and treat microorganisms commensurate with their place in the natural order of things. However, effecting this remedy first requires diagnosis: Scientists need to understand why this view, this unnatural view, of microorganisms exists.

Understanding starts with the realization that without microorganisms macroscopic life (as we know it) would not exist. Macroscopic life is sustained by photosynthetic carbon fixation. Although we tend to associate photosynthesis with plants, its evolutionary origin is in the bacterial world. Plants evolved their photosynthetic capacity only in the sense of acquiring it, through endosymbiotic interactions with (cyano)bacteria. Thus, the main by-product of photosynthesis, our oxygen atmosphere, owes its existence ultimately to the bacteria. The capacity of multicellular organisms to utilize oxygen, which resides in mitochondria, has also been acquired through endosymbiosis, involving what are known as the purple bacteria (2). At all levels bacteria are fundamental to the global ecosystem. Global recycling turns on their metabolism. Oxygen levels aside, the global balance of other important atmospheric gasses, such as carbon dioxide and methane, is to a large extent controlled by bacterial metabolism. Even mineral deposition is to some extent of their doing. In a fundamental sense, the biosphere is the bacterio-sphere.

Contrast this image, this central role microorganisms play in the natural order of things, to the lowly place they hold in our modern-day cloisters, the universities and other scientific institutions. University microbiology departments are withering in two ways: (*i*) we are eliminating them as entities in their own right (usually demoting them to ineffectual "programs" within departments whose names typically begin with "Molecular" or "Cellular"); and (*ii*) within those that remain as Microbiology departments in name, the emphasis in both teaching and research is shifting away from microbiology *per se:* Microorganisms are increasingly studied for either practical reasons (medical, agricultural, and environmental concerns) or as vehicles for approaching problems on the molecular level (as easily manipulated systems in biochemical/molecular analysis and as systems for cloning, sequencing, and expressing genes). The topic of microbial diversity has indeed become an endangered course in microbiology curricula. We scientific monks are shifting away from, not toward, a useful understanding of microorganisms.

Why have we done this; why does this disparity exist between the place microorganisms hold in the natural order of things and our scientific (and societal) perception of them? Obviously, the fact that microorganisms are small has nothing to do with it. Far smaller biological entities are prominent in our everyday imagery—the ever-present DNA double helix, computer-generated models of proteins, detailed renderings of deadly viruses, *Scientific American* style drawings of cell receptors and ion channels, etc. The problem does lie, however, in the microbiologist's concept of microorganisms—actually the lack thereof. What was said long ago by Stanier and van Niel (3) applies today: ". . . the abiding intellectual scandal of bacteriology has been the absence of a clear concept of a bacterium." Nothing learned since has remedied this situation.

Why microbiologists, and, therefore, all of us, are in this unfortunate situation is easy to understand. Technological limitations prevented microbiologists in the past from determining the natural (evolutionary) relationships among bacteria. Imagine a zoology where the evolutionary relationships among animals (which at one level are self-evident) were unknowable. Worse, imagine not being able to tell whether something is even an animal or a plant! Yet that is, or was, the state of microbiology through most of the 1970s. Because of this, bacteria could not be understood in their own right. And their relationships to one another in their natural settings (i.e., microbial ecology) was little more than a simulacrum of the "real" ecology, that practiced for the higher forms. Indeed, microbiologists couldn't even take a representative census of the organisms in any particular niche. (The technique of enrichment culturing, which has been the mainstay and font of microbiology for the better part of a century, failed completely in this regard. For the microbial ecologist, what can be cultured is the basis of his conception of what exists. This is exactly like learning about animals from visiting zoos; their natural representation and their behaviors are completely distorted.)

The good news is that these severe impediments to a true understand of microorganisms have now been overcome. Microbiology today is in the process of developing a meaningful "concept of a bacterium" based upon knowing the natural relationships among the various species and upon new and powerful approaches to microbial ecology. The paper by Barns *et al.* (4) in this issue is a prime example of that new microbiology; and it

represents a milestone in the program started a decade or so ago by the paper's senior author, Norman Pace, a program that is revolutionizing the study of microbial ecology.

The natural relationships among prokaryotes eluded microbiologists because the only microbial characteristics they could determine—cell shape, motility, certain physiological parameters, colony morphology, etc.—were, almost without exception, far too simple or variable to be phylogenetically telling (5, 6). It was not until prokaryotes could be characterized on the molecular level, particularly in terms of molecular structures and sequences, that they became sufficiently complex for us that we could begin reliably to infer their genealogical relationships. As these relationships emerged [in the main from ribosomal RNA sequence characterizations (7–10)], it became obvious that all prior bacterial taxonomies had little phylogenetic validity (except at close range, i.e., within genera). Other surprises were in store, such as the incredible phylogenetic diversity of prokaryotes—by which standard, animals and plants seem closely related. Perhaps the most stunning surprise of all was that the world of prokaryotes, which biologists had taken to be phylogenetically coherent, was not so (7, 10, 11): There exist two groups of prokaryotes, the (eu) Bacteria and the Archaea, which are no more related to one another than either is to the Eucarya. In fact, the Archaea are specific relatives of the eukaryotes (12–14).

Provided at last with a phylogenetic articulating framework, microbiology can now grow to become a complete biological discipline, a discipline infused with a new spirit. Within this phylogenetic comparative context, new isolates are no longer mere anecdotes; they are, rather, pieces in a growing and beautiful evolutionary mosaic. Consequently, isolating new microorganisms has once more become a highly regarded activity. An impressive number of novel genera, families, orders, etc., of prokaryotes have been cultured over the last decade. And yet, one must wonder what microbial gems remain hidden in nature; because they can't be cultivated or, for that matter, even detected. But, we no longer need sit and wonder.

In the early 1980s Norman Pace had a critical insight: He realized that, given a phylogenetic framework, it was no longer necessary to isolate microorganisms in the laboratory to be able to tell something meaningful about them. One needed only isolate one or more of their genes directly from the environment. Determining sequences of, for instance, rRNA genes from some niche could tell you what phylogenetic types ("phylotypes") occupy that niche (15, 16). From these rRNA sequences, specific DNA probes could be designed that would permit microscopic identification of the organisms corresponding to the sequences and, as well, allow a determination of their relative numbers (17). Now, for the first time, an exhaustive census of a microbial niche became a possibility: Microorganisms could no longer evade detection or hide their identity.

One of the more spectacular applications of this direct method for "taking a census" of a microbial niche can be seen in the recent work of E. F. DeLong (18): Many new species of Archaea have been isolated since that group's discovery in 1977 (7, 11), and workers in the field (myself included) had begun to believe that all the major archaeal phenotypes had been identified (10, 19)—i.e., the methanogens, the extreme halophiles, the thermophilic sulfate reducers, and the so-called "extreme thermophiles"—organisms that not only grew at highly elevated temperatures but also had metabolisms centered about sulfur and sulfur compounds. [Note for future reference the fact that one of the two major taxa of Archaea, the kingdom Crenarchaeota (14), was thought to comprise exclusively species of this last type.] Yet, working with marine samples, DeLong (18) was able to amplify and characterize many examples of rRNA genes representing two new major archaeal phylotypes that, given the environments from which the samples were taken, must have phenotypes unique among the Archaea.

The paper of Barns *et al.* (4) in this issue further realizes the potential of this new approach to defining a microbial niche. The work is an extensive, though not yet exhaustive, census of the Archaea in a single Yellowstone hot spring, known as "Jim's Black Pool." The diversity found therein is exceptional—or is it? This is the first time that one (small) niche has revealed members or close relatives of all the known genera of (cultured) Crenarchaeota—a kingdom that has been defined through culturing and identifying many hundreds, perhaps thousands, of isolates from perhaps hundreds of thermal environments throughout the world. The question is whether Jim's Black Pool is uniquely cosmopolitan or whether with techniques of direct phylotypic characterization of a niche (extracting genes, not organisms), one can detect much more than one does by the tried and true method of enrichment culturing.

The evidence favors the latter explanation, for an even more remarkable finding of the study by Barns *et al.* (4) is the detection in abundance of species of Crenarchaeota only remotely related to any that now exist in culture—lineages that greatly deepen the branching of the crenarchaeal tree. Traditional microbiology, which has isolated the known crenarchaeal species time and again by customary methods, is now faced with the challenge of isolating, and the pleasure of characterizing, a number of new and undoubtedly highly novel crenarchaeal species. Fortunately, they will be aided in this by specific DNA probes/primers designed from the rDNA sequences of the new and so-far uncultured species.

The take home lesson from the study of Barns *et al.* (4) and others like it would seem to be that although microbiologists have discovered a rich microbial diversity using the classical method of enrichment culturing (developed by Beijerinck about the turn of the century), they are far from exhausting that richness. Although the direct gene isolation method now fails us by not identifying the actual phenotype of the organism from which the gene has come, the approach more than compensates for this by (*i*) telling us what phenotypically characterized organisms are related to the unisolated one (and how close those relationships are), (*ii*) allowing us to design probes/primers to aid in efforts to isolate the organism in question by enrichment culturing, and, best of all, (*iii*) having the potential for a complete accounting of the microbial species in a niche (except perhaps for organisms occurring at relatively low levels). Pace's method of exhaustive phylotypic characterization of a niche is in a real sense the complement to Beijerinck's method of enrichment culturing. Together, as opposite sides of the same coin, the two approaches give microbiologists the power to define, understand, and revel in the full richness of the microbial world. This is the new dawn that a phylogenetically based microbiology brings.

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