Capturing and taming Earth's wild microbes

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Among the organisms of the biosphere, the diversity of the Bacteria and Archaea is the most poorly understood. Our lack of knowledge about microbial diversity is important because the majority of these organisms still remain undescribed and unclassified and the roles they perform in their environments, their geochemical activities and their biotechnological potential still need to be ascertained. For these reasons, this rich, unknown diversity comprises an enormous untapped resource for science and society. Only recently have microbiologists begun to more fully realise how great our ignorance of microbial diversity truly is. This paper discusses how technology, persistence and serendipity play important roles in unveiling the vast diversity of uncultivated microorganisms through their capture (isolation) and taming (cultivation, naming and description). Perhaps citizens can be recruited to become 'microbe hunters' to assist in efforts to characterise new microbial species. Their potential reward could be a trophy for the 'smallest game': the capture and naming of a novel bacterial species.

Our ignorance of microbial diversity began to be more fully appreciated in the 1970s and 1980s with the realisation that a daunting difference existed between the numbers of bacteria that could be cultivated from the environment in comparison with the total numbers that were there. Although microbiologists had traditionally used plating procedures for enumerating the "viable" bacteria from natural habitats, the poor recovery of organisms was not recognised until a total microbial counting procedure, a direct microscopic count for Bacteria and Archaea, had been perfected, the acridine-orange, fluorescence counting procedure[1]. The quantitative difference between the best cultivation medium available was a small fraction, typically less than 1%, of the total bacteria from many environments. Notably, this disparity was most apparent in oligotrophic environments in comparison with eutrophic ones (for example,[2]). This significant discrepancy was termed the Great Plate Count Anomaly[3].

The introduction of molecular approaches to microbial diversity studies brought an appreciation of this difference from a qualitative perspective: the difference between the microbial species that could be readily cultivated compared to those that could not. This major advance began with the construction of the Tree of Life, indicating that Bacteria and Archaea comprised two of the three domains of life on Earth[4]. This in turn led to the phylogenetic classification of the Bacteria and Archaea in *Bergey's Manual*[5] that included the description of members of known phyla based upon 16S rRNA gene sequences available from existing pure cultures. Accompanying this advance, PCR procedures using universal primers for 16S rRNA genes were applied to DNA that was extracted from environmental sources.

The resultant 16S rRNA gene sequences that were recovered from various environments revealed that entirely new bacterial and archaeal phyla existed[6] and not a single bacterial isolate had been cultivated, described, named and deposited in culture collections from these novel groups. Some have estimated that in addition to the original dozen or so prokaryotic phyla from which cultures were available, another 100 or more phyla reside in natural environments that still have no cultivated representatives. So, although technological breakthroughs have led to a fuller appreciation of the qualitative as well as the quantitative importance of uncultured bacteria in comprehending microbial diversity, much work still remains to be done.

Cultivation

A variety of new cultivation strategies have been recently developed. Jared Ledbetter[7] has written an excellent review on this topic. For example, high-throughput methods using dilute media or water from natural habitats resulted in isolating new taxa such as members of the SAR 11 clade from the marine environment[8,9]. Zengler *et al*.[10] developed a method in which cells from an environment were encapsulated in gel microdroplets, incubated under different conditions and the droplets separated by flow cytometry to detect those in which cells had grown. Those exhibiting growth were then further studied by additional cultivation and characterisation.

Enhanced recoveries of soil taxa including previously unknown members of the *Acidobacteria* and *Actinobacteria* phyla were obtained from pasture soil using a dilute medium amended with a polymeric carbon source, xylan, following extended incubations[11]. Likewise, representatives of poorly understood, microbial phyla including the Verrucomicrobia and Acidobacteria were successfully cultivated from soil and termite guts using an integrative approach that entailed the use of low-nutrient media supplemented with various additions such as humic acids and quorum sensing compounds coupled with a variety of lengthy incubations using low-oxygen concentration or anoxic conditions[12].

The discovery of a single isolate from a new phylum constitutes a breakthrough in our understanding. An example of this is the cultivation of a member of the phylum Gemmatimonadetes. *Gemmatimonas aurantia* is a heterotrophic, rod-shaped bacterium that divides by budding as well as binary transverse fission. A colony of a representative of this phylum was discovered on a Petri plate that had been inoculated from a phosphate-limited enrichment medium derived from a wastewater treatment reactor[13].

Another example is the recent description of members of the new phylum, *Caldiserica*, from a hot spring in Japan[14]. The new species, *Caldisericum exile* is a representative of the OP5 group that was first reported in gene libraries from Obsidian Pool in Yellowstone National Park[6]. The new species is a filamentous, obligately anaerobic, thiosulfate-reducing organism.

The examples mentioned above illustrate the roles that imagination, persistence, patience and serendipity, in addition to technology, play in the discovery of novel microbial life forms. Once a single isolate has been discovered from a novel phylum, its features including habitat, growth conditions, metabolic and physiological features serve as a starting point in designing conditions for the isolation of additional representatives of the phylum from the same and similar habitats. These new strains may have significantly divergent properties and taxonomies.

Microbial game hunting

The vast diversity of microbial life on earth may be in the millions or even billions of species and, therefore, beyond the capacity of professional microbiologists to isolate and describe them all. The accomplishment of this goal calls for totally different approaches and models. Also, governmental granting agencies have not been very supportive of proposals for microbial systematics research in contrast to culture-independent methods. However, it may be possible to develop an international effort to fund a program that explores the biodiversity and biogeography of microorganisms. Another idea that may be worth considering is the initiation of a global effort, analogous to volunteer 'earthwatch' efforts, to recruit the help of 'microbe hunters' from citizens who have a nominal knowledge of science and who are entranced with the idea of assisting in the study of microbial biodiversity. Perhaps private laboratories or university training facilities could be established and supervised by professional microbiologists that would charge a set fee, comparable to that needed for an African safari or a Galápagos Islands tour, for microbe hunters to join in the effort to isolate, name and describe novel organisms. Applicants would have to be screened to identify qualified individuals. They could then go to a facility that has the instrumentation necessary for the isolation and description of novel species and join a laboratory that is working on a particular group of organisms of their interest. This approach might fulfil the interest of citizens to act in a way that is analogous to that of the "parabiologists" who have worked on plant and animal biodiversity efforts. Furthermore, the potential reward or 'trophy' for these 'smallest game hunters' could be a contribution toward the naming of a novel species. This could become the ultimate way for citizen scientists to participate in microbial eco-tourism.

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Biography

Jim Staley is an emeritus professor from the University of Washington in Seattle. His research interests include biodiversity, microbial ecology and bacterial taxonomy. Current activities involve studies of nitrogen cycling activities in the Black Sea, the pursuit of a universal species concept and serving as editor of the new *Bulletin* of Bergey's International Society for Microbial Taxonomy (www.bergeys.org). He and his family enjoyed a sabbatical leave in Australia in Kevin Marshall's and Vic Skerman's laboratories in 1977–1978.