MICROBIAL ECOLOGY

Variation among the masses

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ANALYSIS of the composition of natural microbial populations is often restricted because the component organisms have to be grown in the laboratory. But by comparing molecules from the microbial environment, rather than the organisms themselves, this need for cultivation can be circumvented. In reports appearing on pages 60 and 63 of this issue, Giovannoni *et al.*[1] and Ward *et al.*[2] describe the characterization of the 16S ribosomal RNA (rRNA) sequences from naturally occurring biomasses using broadly applicable techniques. Although the groups examined entirely different environments, and used different procedures, the most important observations to emerge are strikingly similar. First, none of the organisms revealed by the molecular analyses are the same as any of those cultivated from the corresponding, or similar, environments, showing that the diversity in the natural populations is far greater than that demonstrated by cultivation-based methods. Second, the microbial populations seem to include distinct clusters of closely related species.

Distinctive characteristics

The identification of a microorganism requires the detection of its distinctive array of characteristics, a process that has traditionally required pure cultures. In some cases the detection of a particular molecule (for example an unusual lipid or chromophore) can be used as a marker for a specific group of organisms in a mixed population. But the availability of suitable markers is limited and they do not necessarily have the desired phylogenetic resolution.

In 1965 it was pointed out[3] that the sequence of a nucleic acid or protein is much more evolutionarily informative than is knowledge about whether its activity is present or absent (usually inferred from the presence of a corresponding metabolic product). The identification of specific macromolecular sequences in natural biomass therefore provides a more precise and powerful characterization of a population than do inventories of lipids or chromophores. Sequences allow a quantitative definition of both the genetic diversity and the organismal relationship within a niche.

The use of ribosomal RNAs in identifying organisms and inferring their natural relationships, a field pioneered by Woese, has been especially valuable because these molecules are universally distributed, constant in function, slow to change in sequence and easily manipulated[4]. Ribosomal RNAs can also be used to characterize uncultivated organisms[5]. Pace and colleagues recognized the potential of rRNA-based analysis of natural populations[6]. Early studies based on 5S rRNA demonstrated the principles, but were limited by the need to purify each molecular species and by the small size of 5S rRNA (about 120 nucleotides)[5-7]. Use of the more informative 16S rRNA (about 1,500 nucleotides) or 23S rRNA (about 2,900 nucleotides) came with the recognition that rRNA-specific primers could be used to sequence the larger molecules rapidly[8,9]. A final obstacle, the searching of large libraries of cloned DNA from natural populations for rRNA genes, was overcome by applying rRNA-specific primers to the amplification of the desired sequences from bulk genomic DNA before cloning[10].

Ward and colleagues[2] have now examined the 16S rRNAs from a well-studied photosynthetic microbial mat in a hot spring in Yellowstone National Park. Previously, the authors had sequenced the rRNAs from an assortment of bacteria cultured from the mat. In the new study, the 16S rRNA molecules from mat biomass were selectively copied (using a 16S rRNA-specific primer) into DNA, which was then inserted into a bacterial plasmid. Subsequent clonal purification of the plasmid replaced the traditional culture-based purification of the microorganisms. Eight distinct sequence types were represented in the 15 plasmid clones characterized. None of these rRNA sequences is the same as those from any of the 12 cultured prokaryotes believed to be associated with the mat. More significantly, in only one case does a sequence bear any close resemblance to a recognized bacterial 'phylum'[4]. Unless the bias in the organisms represented by the cultures and the bias in the rRNA-based characterization are in opposite directions, it would seem that the species represented in culture are but a small fraction of the organisms in the mat.

Giovannoni and colleagues[1] looked at the 16S rRNA gene sequences of organisms from a sample of oligotrophic (low-nutrient) ocean water. The authors used the polymerase chain reaction to amplify the 16S rRNA gene sequences in their sample of bulk genomic DNA before cloning the DNA into a bacterial plasmid. The nine sequences obtained fall into two clusters, one related to cyanobacteria (oxygen-producing photosynthetic bacteria) and one to another main eubacterial group, the alpha purple bacteria[4]. Each cluster contains three distinct sequences. Although this work is not directly related to a culture-based study, D. Distel and J. Waterbury of the Woods Hole Oceanographic Institute have determined 16S rRNA sequences of cyanobacteria isolated from similar environments (see ref. 1). The cultured cyanobacteria are not the same as any of those represented in the environmental samples, but two of them are clearly members of the same cluster (raising the number of distinct sequences in the group to five).

Natural diversity

The tentative conclusion that can be drawn from the two latest findings is that cultured organisms represent only a small proportion of the diversity in nature. Giovannoni *et al.* also emphasize the sequence diversity within their two clusters. Ward *et al.* uncovered seven disparate groups, only one of which has more than two sequences in it. This last group also shows sequence diversity (about three per cent sequence difference) comparable with that observed by Giovannoni *et al.* For perspective, this is comparable to the difference between the 16S rRNAs of *Escherichia coli* and *Salmonella typhimurium*.

These studies mark the advent of a new stage in microbial ecology. Neither group routinely determined complete 16S rRNA sequences, and so the precision of the inferred sequence relationships is not certain. Also, the sampling of population diversity is too small for confidence in the apparent trends; confirmation of the results might require the characterization of many more clones.

In the near future, selected microbial populations will no doubt be thoroughly investigated with molecular techniques. The resulting data will be essential for understanding such processes as genetic divergence, physiological divergence, migration and competition in the microbial world. In addition, the molecular data are directly applicable to the design of rRNA-based hybridization probes[1] and 'phylogenetic stains'[11], which can be used for more easily tracing the distributions of bacterial species or groups of species. □

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