Environmental biotechnology
Better living through biochemistry

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Abbreviation
PHA poly(hydroaclkaoate)

Believe it or not, new insights into microbial ecology have begun to provide benefits even a conservative curmudgeon can appreciate. Under its new fashionable name of environmental biotechnology, the 12 reviews in this issue will define some currently practical, and also hugely potential, benefits of this research. The first two contributions define the new technologies and their important limitations and the rest illustrate practical applications in widely diverse fields.

It has been the cardinal contention of this laboratory for 30 years that insight into the various interactions of microbial communities as systems is essential in understanding the biosphere. Although these microbial communities are by far the largest biomass, with the most diverse metabolic prowess in every biome, they have been largely ignored by funding agencies that have concentrated on the ecology of organisms that could be readily observed. To examine the silent majority, methods of assessing microbial communities independent of isolation and growing the organisms as monocultures were necessary. Isolating organisms from their communities could be described as 'hand grenade' biochemistry — the assumption that what organisms do as isolated monocultures reflects what they were doing before the 'hand grenade' of isolation is fundamentally flawed. The discovery of a wealth of currently uncultured-organisms, some of which may not be capable of growth in isolation, emphasizes the poverty of our understanding of microbial community function in the environment.

Initially, we chose to utilize lipid biomarkers to examine the total community, as they had sufficient diversity to be useful taxonomic markers yet had molecular weights that made them structurally accessible at required sensitivities by the newly emerging gas chromatography/mass spectrometry. Unforeseen benefits from examination of the lipids were derived. As all living cells have an intact membrane containing polar lipids, cell lysis induced by insults that do not immediately denature proteins allow phospholipases to form diglycerides from the polar lipids. Thus, polar lipids provide information regarding the viable biomass, while the diglyceride fatty acid patterns describe the readily lysed components of the community. Although the detailed analysis of the lipid components does not provide differentiation of each species in the community, it does provide indications regarding groups of organisms, which has proved very useful [1], as well as a second unforeseen benefit — insight into the nutritional/physiological status of the community. Specific groups of organisms react to specific environmental stresses with structural modifications of their lipid composition [2]. Consequently, lipid biomarker analysis provides deep and quantitative insight into the viable biomass, cell lysis, community composition, and nutrition/physiological status of the community under examination. As communities respond to their microniche environment it is possible to utilize the community response for quantitative toxicity and risk analysis assessment [3].

Lipid biomarker analysis has severe limitations, however, in differentiating many taxa and groups of taxa. Developments in the analysis of microbial communities targeting ribosomal RNA, at the moment almost exclusively 16S homologues, have arisen to partially compensate for this void. Originally, microbial community 16S analyses utilized laborious total-community DNA cloning and hybridization methodologies. This approach was rapidly superceded by the introduction of PCR-based methods to amplify these genes specifically, thus eliminating the hybridization steps. These methods remained too costly and time-consuming, however, to permit detailed comparisons of communities or to monitor changes over time in response to environmental change. The introduction of cloning-free methods to analyze PCR-amplified ribosomal gene fragments, starting with denaturing gradient gel electrophoresis [4], represented a major break-through for microbial ecology and the field of environmental biotechnology. Our laboratory has shown that the combination of lipid and rDNA analyses can be used to make predictions in the disturbed environments associated with bioremediation [5]. So there is now a quantitative handle on the microbial community. Can we progress to the latest rage — estimation of the microbial community biodiversity and estimate the benefits of maintaining biodiversity? PCR-based methods all have one problem in common. Although they may reveal a greater diversity than traditional culturing methods, current approaches do not detect numerically minor components of the target community, where the bulk of the diversity of a community may well abide. This is, in part, a function of the sensitivity of current gel-based methodologies, which may be partially alleviated by the introduction of chip technology and microarrays. Can we define metabolic activity and potential metabolic activity? Not very well — what we are learning is that in the environment microbes can do nothing for long periods and pounce on substrates with extraordinary rapidity. There are a few challenges left!

We start the issue with a scholarly review by MacGregor (pp 220–224) who carefully defines the promise and shortcomings of the molecular revolution and suggests how combined approaches can be used to make the connection between microbial diversity and function. This is a comprehensive treatment of this fast moving field emphasizing fresh-water sediments as the environment of specific interest. Soil, the most complex environment in the biosphere, is slowly yielding insights by the proper application of molecular methods. The problems and possibilities of opening the 'black box' of soil microbial communities are discussed by O'Donnell and Görres (pp 225–229) in the second paper of this issue. Metal and nuclide contamination of the subsurface environment provide a difficult challenge to bioremediation as the contaminants are nearly immortal and are often combined with organics to form mixed wastes. Manipulations of subsurface microbiota for solubilization or immobilization by use of nutrient amendment, metal chelators, genetically altered bacteria, constructed wetlands, and community bioprotectant species are discussed by Stephen and Macnaughton (pp 230–233). Head and Swannell (pp 234–239) discuss advances in the bioremediation of hydrocarbon contamination of the marine environment, which despite the advances in molecular analysis, remains essentially an empirical technology. They see resource-ratio theory as a theoretical framework that may make the effectiveness of both aerobic and anaerobic bioremediation more predictable and effective in destroying the most toxic components of the spills. Isolates primarily from the Antarctic that can provide polyunsaturated fatty acids (PUFA), cold-adapted enzymes and bioremediation prowess in cold environments are discussed by Nichols *et al.* (pp 240–246). The magic microbes from the Australian Collection of Antarctic Microorganisms have provided a host of new species with commercial biotechnological potential. Particularly exciting are the PUFA recovered from previously undescribed taxa within the genera *Shewanella* and *Colwellia*. With the rapidly declining stocks of fish these organisms and their genes for the biosynthesis of essential human nutrients will be of ever increasing importance.

Plants may be the ultimate parasites. With chloroplasts from *Cyanophytes*, mitochondria from bacteria, and mycorrhizae from fungi they are enormously successful and are the backbone of the biosphere. In an elegant article, Kowalchuk (pp 247–251) shows us that without molecular methods, progress in understanding the fungal–plant interactions was enormously inhibited and the rational interpretation of ecological experiments was far less detailed. The use of these molecular methods has not only provided phylogenetic insights but has also made possible manipulations that could increase the productivity of food and fiber on which our civilization depends. The biopolymers that the plants make are the source of paper, another essential component of our modern world. In an article describing the economic and environmental driving force behind recent advances in biopulping, Breen and Singleton (pp 252–258) show that bioprocessing by a complex of enzymes can make a significant impact on lowering the energy costs of making paper. Economic factors as well as environmental constraints are making enzymatic means of separating the cellulose from the lignin ever more practical.

Plastics are another essential component of our world but one which poses problems: firstly, for disposal in composting or landfill with their resistance to biodegradation; and secondly, in the eventual shortage of basic petroleum precursors. Witholt and Kessler (pp 279–285) show that the synthesis of myriad poly(hydroxyalkanoate) (PHA) polymers by bacteria can provide a renewable source of plastics with controlled biodegradability. The genes for the synthesis of PHA can be transferred in microbes and ultimately to plants at ever-increasing effectiveness. Indeed, it may be possible to grow PHA-based plastics as agricultural crops in the not too distant future. Maintenance of the structure and metabolic function of microbial communities within wastewater treatment facilities is vitally important to reliable remediation and containment of civilization's effluents. Recent advances in the understanding of the roles of exopolysaccharides in serving this function are discussed by Houghton and Quarmby (pp 259–262).

The biofilm form of microbial growth can be examined utilizing confocal microscopy at the single cell level. Palmer and Sternberg (pp 263–268) describe how biofilm architecture, species composition and metabolic activities can be directly monitored in four dimensions. Localized mapping of biofilm microbial dynamics and the effects of heterogeneity promises to be an exciting area of environmental biotechnology. One of the consequences of localized activities in biofilms is microbially influenced corrosion (MIC). Angell (pp 269–272) describes how microbial metabolism locally changes the electrochemical environment, which can markedly affect corrosion. Research on mechanisms of MIC is providing new insights into manipulations beneficial to control of this biofilm biotechnology. Contamination of food is an increasingly important problem. Mandrell and Wachtel (pp 273–278) review recent progress in rapidly identifying *Salmonella* and *Campylobacter* spp. Contamination in poultry using genetic, immunomagnetic separation techniques and time-of flight mass spectrometry.

In summary, these are exciting days in environmental biotechnology. Applications of new methods are spreading into ever-more diverse applications and gaining commercial acceptance. The 'black boxes' that were the actively remediating communities are giving-up their secrets and becoming more amenable to manipulation. Biofilm structures and dynamics are being elucidated, the first step towards cracking their currently 'invincible' status. We can look forward to a cleaner, healthier environment through the application of the discoveries outlined here and the continued application of molecular tools.

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