within some members of the earth's diverse microbial population.

How will these new metabolic reactions be discovered? By defining what functional groups will undergo metabolism, the reactions they undergo will sometimes be predictable using organochemical logic. Thus, the reactions can be searched for systematically by establishing logical selection or screening methods. Searches will increasingly be accomplished without isolating pure cultures of bacteria from an environmental source. Gene libraries will be prepared from total prokaryotic DNA present in a soil, for example, and then the desired reaction will be identified by a high-throughput screen. In specific cases, the enzymatic reaction or biochemical pathway will be exploited for biotechnological gain. The biofabrication of silicon structures for electrical devices is just one example of this. A larger biotechnological toolkit of enzymes will drive other applications. In many cases, the natural enzyme will transform the desired chemical group in a desired way but lack the required specificity for the overall structure of the commercially relevant molecule. Laboratory evolution methods, such as DNA shuffling, will then be used to modify the specificity of the enzyme to catalyse the reaction efficiently with the biotechnologically correct substrate.

When humans explored their local environments several millennia ago, maps could be two-dimensional, like current metabolic maps. As the face of the earth was explored, it became necessary to visualize its entirety using a globe, although two-dimensional maps, with all their territorial distortions, continue to be used for convenience. As we map the world of microbial metabolism, we will increasingly go outside the boundaries of the current metabolic maps. With computers, we can go past three dimensions to appropriately map metabolic networks. Undoubtedly, this will be done; for genomics, for biotechnology, for a better understanding of the world of microbial metabolism.

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Microbial community analysis

David C. White, University of Tennessee

It is an honour to be asked to look into the future by Ken and Dave. The future will bring new technology making possible deeper understanding of environmental microbiology. This understanding will come from leaving the era of reductionist biochemistry in which monocultures were snatched from their environment, incubated in rich media to promote cell yield then exploded into their enzymes and cofactors to see how they worked. From this, what we called 'hand grenade', we were to reconstruct the interactions that cells had in their environmental communities. We now know that we isolated only a few of the organisms, and in many cases our laboratory strains lost essential components of their enzymatic repertoires. It was a hopeless task. Then, came the magnificent sequencing of whole genomes when we found that the majority of genes had unknown function in most of the bacteria examined. About this time came the realization that the 'biofilm way of life' with its slow protected low-gear-resistant metabolism was the status of most microbes certainly in the terrestrial environment. The genes active in this biofilm living accounted for many of the unknown functions in the whole sequence. Recently, environments with high species diversity such as grassland, marine ecosystems as well as microbial chemostat methanogenic microcosms, have been shown to form communities able to withstand changes in the environment maintaining stable functions, although the occurrence and abundance of particular species and strains may vary greatly. The metabolic tasks of disappearing communities are taken over by a succeeding group with different community components. Recently, this appears also in dental plaque communities in which C. H. Sissions of New Zealand has shown the resting pH of plaque microcosms is stable parameter. This group has devised an elegant system for forming multispecies biofilm plaque communities that undergo succession with reproducible community shifts as a function of pH and time(1). The formation of biofilms, particularly when a readily modifiable driver induces reproducible successions, provides the system in which to study interactions between components in biofilm communities. There are now emerging methods to characterize these communities, both in terms of components and activities.

It has been particularly gratifying to us that the lipid biomarkers including both neutral lipids (respiratory quinones, sterols and digycerides) and polar lipids provide quantitative insight into viable biomass and community composition, as well as physiological status that relates to community function reflecting the organization of cellular membranes to cope with a changing environment. Biolog analysis provides an index of functional diversity. Checkerboard DNA:DNA hybridization analysis, terminal restriction fragment length polymorphism (T-RFLP) analysis, and polymerase chain reaction (PCR) of rDNA and functional genes provide detailed ecological insights of the complex communities. Preliminary investigations show

that functional analyses of proteins analysed with 2D gel, despite the complexities of the communities showed at least 32 proteins (spots), were consistent in the communities maintaining different pH, at least 17 new spots appeared and at least eight spots disappeared as the community shifted to one maintaining a pH of 4. A further level of organization results from the heterogeneity of organism distributions when observed with the confocal microscope. These plaque systems self-organize under controlled conditions. This heterogeneity is further compounded by differences in the function of specific components in space and time. Newly developed fluorescent precipitates on individual cells as result of hydrolysis of non-fluorescent substrates allow analysis of the distribution of activities in the biofilm (2). Not all the capable cells actually appear to function at a given time. Is there altruism in the microbial world? We are developing the tools to find out.

Quorum sensing in monoculture biofilms has begun to yield insights into population dynamics. We are now poised to begin to examine more interesting interactions in the communities in which microbes actually function.

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The future for culturing environmental organisms: a golden era ahead?

Stephen H. Zinder, Cornell University

The late 20th century was a tough time for cultural microbiology in the USA (less so in Europe and elsewhere). A few anecdotes follow. When I was a beginning Assistant Professor in the early 1980s, a prominent microbiologist advised me that I would never be considered a 'heavy hitter' if I only isolated and characterized novel organisms, and that I needed to do biochemistry or molecular biology. A colleague recently told me that in the 1980s a funding programme officer consulted him about whether a grant proposal that featured isolation of an organism was too risky despite the strong track record of the PI, because isolation could not be guaranteed. Moreover, concerns were raised by many microbial ecologists about the relevance of pure culture studies to natural populations, and in a 1994 letter to *ASM News* Doug Caldwell proclaimed an end to the era of pure culture microbiology.

In the 1990s, 16S rRNA-based techniques rightfully grabbed centre stage in microbial ecology, and isolation became even less fashionable. These studies confirmed the already suspected notion that only a miniscule fraction of the microbial diversity present in most natural habitats had been cultured. Based on studies on poor recovery of pathogens from some habitats these 'uncultured' organisms were pessimistically deemed 'uncultivable' by many. Indeed, I found myself reviewing grants that spent several pages detailing molecular methods to study microbial populations but only a few cursory sentences, if any, on cultural studies on the organisms possessing those sequences. In one case, the PI said that the superficial and poorly designed cultural studies would demonstrate that the organisms in question were not cultivable. I've also heard it stated that culture isn't really needed, that one can simply sequence the metagenome of a habitat to know everything about it. I believe that the frequent use of the term 'uncultivable' had a chilling effect on considerable efforts needed to culture organisms from natural habitats.

However, this crisis carried the seeds of the revival for cultural microbiology. Successes began to occur in culturing environmentally significant organisms identified in molecular studies. For example, David Ward and colleagues cultured cyanobacteria present in significant numbers in hot spring microbial mats by serial dilution instead of enrichment from undiluted material, which selected for the fast growing but less numerous *Synechococcus lividus*. In an application of Robert Hungate's principles of habitat simulation, Donald Button and colleagues performed most probable number counts of marine bacteria by dilution into unsupplemented sterilized seawater in which positive tubes containing only 10^4 bacteria per ml were detected using a fluorescence-activated cell sorter. This technique yielded viable counts approximately 60% of direct microscopic counts. Whereas very low nutrient concentrations (5 mg l^{-1} casamino acids) were inhibitory to these organisms directly from seawater, they were less inhibitory to the cells in the MPN tubes, allowing isolation. In a final example, using inferences from molecular and ecological data (and a great deal of persistence), Karl Stetter and colleagues succeeded in culturing *Thermocrinus ruber*, the pink bacteria that used to mock me by growing in 'uncultivable' thick masses in hot springs when I was a graduate student working in Yellowstone National Park.

There have been numerous other successes in culturing microorganisms, and I am beginning to sense greater interest in culturing organisms identified in molecular studies and a more optimistic attitude that this can be achieved. Indeed, I now see the perhaps overly optimistic 'not-yet-cultured' used instead of 'uncultured' in some papers. Moreover, there is greater appreciation of the