

has generated a two-class distinction of sub-fields of microbiology. Sadly, ecology has been largely relegated to the less desirable class by many of those who study solitary bacterial life because they find the types of evidence and structure of arguments in ecological study to lack the precision to which they are accustomed. All of that can change.

Consider the future

Metagenetics will dissect ecological questions at a new level of precision. But unlike the early days of bacterial genetics, the new field of metagenetics will be buttressed by vast databases of sequence from metagenomic analysis. Metagenomics will generate hypotheses to be tested with genetics as well as the sequence information on which to base mutant construction.

Metagenetics will need to embrace both random mutagenesis, which is a way of giving voice to the bacteria, and directed mutant analysis, which is driven (and limited by) the imagination of the investigator. In the first, we will mutagenize a pure culture and then screen the random mutants for a community phenotype. In the second, we will create a defined mutant in a gene of interest and determine its phenotype in the community context. We might, for example screen a randomly mutagenized population of bacteria for the loss of ability to invade a community or we might construct a mutant lacking flagella and determine whether it is affected in the ability to invade. Both approaches will be facilitated by available genome maps, sequence information and extensive ‘-omics’ (transcriptomics, proteomics and metabolomics) data.

Metagenetics on culturable community members may not seem all that different from classical genetics except in the nature of the phenotype tested. But the bold advance will issue from development of genetic tools to study unculturable members. For example, imagine the power of knocking out all homologues of a particular gene in all members of the community? What would happen if we knocked out all of the polysaccharide biosynthesis genes in a community? Alternatively, what if we knocked them out only in one family of bacteria? Highly specific conjugal vectors and sequence-based homing devices can make these approaches reality. Metagenomics will furnish the raw material for such studies – sequence information from the unculturable members of the community that will form the basis for generating hypotheses and genetic devices to make targeted changes.

Metagenetics, in concert with the other tools of the ecologist, including statistics, modelling, microscopy, radioactive labels, chemical analysis and meta-omics, will elevate the level of rigor and precision with which we can approach community-level microbial ecology. Just as

early bacterial genetics provided critical data beyond microbiology, to the entire field of evolution, microbial metagenetics may advance the entire field of ecology by answering questions at a level and with tools that are not possible in macroecological systems. The lessons from 50 years of bacterial genetics are powerful. Perhaps 50 years from now we will be reflecting on the advancement of ecology by a parallel metagenetic approach.

Future shock from the microbe electric

Derek R. Lovley, Department of Microbiology, University of Massachusetts, Amherst, MA 01003, USA.

How can the future not look bright when you are dealing with a microbial process that can power a light bulb? The study of microbial fuel cells and, more generally, microbe–electrode interactions is rapidly amping up, not only in power production, but also in the number of investigators and areas of study.

The most intense focus has been on wastewater treatment and this is likely to continue for some time. It was probably safe to say 5 years ago that any compound that microorganisms can degrade could be converted to electricity in a microbial fuel cell, but if there was ever was any doubt, this point has been proven over and over again in a plethora of recent studies. It is clear from this work that a major limitation in converting complex wastes to electricity is the initial microbial attack on the larger, difficult to access molecules, just as it is in any other treatment option. It may well be that the intensive focus on the degradation of complex organic matter in other bioenergy fields will soon make a contribution here.

However, there are other issues specific to microbial fuel cell technology. At present the rate that even simple organic compounds can be converted to electricity is much too slow for practical wastewater treatment. For example, coulombic efficiency (i.e. the percentage of electrons available in the organic substrate that are recovered as current) is often diminished by methane production, indicating that even relatively slow-growing methanogens are competing with the current-producing microorganisms. This is despite the fact that electron transfer to oxygen, the ultimate electron acceptor in microbial fuel cells, is much more thermodynamically favourable than methane production.

Some contend that the limitations to current production in waste treatment can be solved with improved engineering of microbial fuel cell design and that there is little need to focus on the microbiology of microbial fuel cells for waste treatment because as better fuel cell designs are developed, the appropriate microorganisms will naturally colonize the systems and produce more power. That may be, but it also seems likely that, going forward, the mechanisms for microbe–electrode interactions will become

better understood and this could significantly inform optimal microbial fuel cell design.

Furthermore, it is likely that we will find that it is possible to greatly increase the current-producing capabilities of microorganisms. This is because there has been no previous evolutionary pressure for microorganisms to optimally produce current. Many of the microorganisms that function best in microbial fuel cells are dissimilatory Fe(III)-reducing microorganisms, which have evolved to specialize in extracellular electron transfer to insoluble, extracellular electron acceptors. However, microorganisms reducing Fe(III) in sedimentary environments are typically in direct contact with the Fe(III). In contrast, when microorganisms are producing high current densities in microbial fuel cells, only a small fraction of the microorganisms in the anode biofilm are in direct contact with the anode surface. Most must transfer electrons over substantial distances through the biofilm. It is not clear that there has ever been substantial selective pressure on microorganisms for such long-range electron transfer. Thus, there should be ample room for improvement.

Another unnatural request that we make on microorganisms when they are asked to generate high current densities is the requirement to metabolize organic compounds very rapidly. The natural habitat of most of the microorganisms that have been shown to be most effective in current production is the subsurface or aquatic sediments. These are rather low-energy environments in which there has probably not been much selective pressure for rapid growth and metabolism. Other challenges to anode-reducing microorganisms include the necessity to tolerate the low pH that can develop within the anode biofilm. This results from the fact that protons as well as electrons are released from organic matter oxidation.

Strains that can better respond to these unusual demands of high density current production will certainly be found or developed. Understanding what characteristics of these strains confer enhanced current-production capability may aid in fuel cell design and these strains may be beneficial in some applications. Strain improvement may include attempts to select better strains from complex microbial communities as well as genetic engineering and adaptive evolution approaches. Some degree of strain selection has taken place in previous studies in which conditions conducive to high current densities have been established in microbial fuel cells and the systems have been inoculated with sewage or some other complex community. The surprising result from a number of laboratories is that such conditions frequently select for *Geobacter sulfurreducens*, or closely related strains. Pure cultures of *G. sulfurreducens* can produce current densities as high as any known pure or mixed culture. We have had moderate success in genetically engineering strains of *G. sulfurreducens* for higher rates of respiration and

extracellular electron transfer, guided by a genome-scale *in silico* metabolic model. However, electron transfer to electrodes appears to be a complex process, and may not be well enough understood to rationally engineer. Adaptive evolution has proven to be a much more promising approach for strain development and major enhancements in power production with this tactic are forthcoming.

As with any optimization procedure, once one bottleneck is relieved another emerges. As better current-producing strains of *G. sulfurreducens* have been developed, it has been necessary to use exceedingly small anodes relative to cathode area in order to keep reactions at the cathode from limiting rates of electron transfer at the anode. The ability of microorganisms to accept electrons from a cathode to support anaerobic respiration has already been demonstrated and studies in a number of laboratories have found that aerobic cathodes selectively enrich for specific microorganisms that might promote faster rates of electron transfer from the cathode to oxygen. This is likely to be an area of intense interest in the near future. It will probably be possible to develop microbes with superior capabilities for accepting electrons from cathodes with the reduction of oxygen with approaches similar to those discussed above for improving the current-producing capabilities of anode-reducing microorganisms.

What if engineering and microbiology do not overcome the barriers to making microbial fuel cell technology suitable for wastewater treatment? There are many other potential applications for microbe–electrode technology. One near-term application is harvesting electricity from waste organic matter or vegetation to power electronics in remote locations. Sediment microbial fuel cells that power monitoring devices at the bottom of the ocean are already feasible. Self-feeding robots that run on microbial fuel cells have also been proven in prototype. There are many other applications in which relatively low power requirements can probably be met with microbial fuel cells. For example, there are already several organizations planning to distribute in developing countries inexpensive microbial fuel cells that run on wastes and can provide lighting or charge electronic devices. A number of research teams are working on developing implanted medical devices that use blood sugar as a fuel. It seems likely that many other applications that require low levels of electrical current but for which it is difficult to install or continually replace traditional batteries could be helped with microbial fuel cell technology. Future applications may also include microbial transistors, circuits and electronic computing devices, among others.

Environmental technology is likely to be another emerging field for microbe–electrode interaction applications. Anodes are attractive electron acceptors for stimulating the degradation of contaminants in the subsurface

because they can be emplaced as a permanent, high-potential, electron acceptor and can adsorb and concentrate many contaminants to co-localize pollutants and the electron acceptor. Current produced from electrodes deployed in anoxic subsurface environments is likely to prove to be a good proxy for estimating rates of microbial metabolism in those environments. Cathodic reactions are also likely to see more application in bioremediation and waste treatment. The potential for stimulating microbial reduction of nitrate, U(VI), and chlorinated contaminants with electrodes serving as the electron donor has already been demonstrated and field application of these technologies are on the horizon.

One of the most exciting areas of future research is almost certain to be the production of specialty chemicals with cathodic microorganisms accepting electrons from an electrode. Fixation of carbon dioxide and its conversion into useful organic commodities powered by electrons supplied directly from an electrode may prove to be one of the most lucrative applications of microbe–electron interactions in the near future. This process is clearly thermodynamically feasible, and the ability for microorganisms to accept electrons for anaerobic respiration has already been demonstrated. It just remains to be seen whether the appropriate microorganisms for this application exist in nature or whether extensive metabolic engineering will be required.

In summary, it would be shocking if the continued increased intensity of study on microbe–electrode interactions did not shed light on additional applications as well as illuminate more of the basic mechanisms by which microorganisms electronically interact with electrodes. The future of this biotechnology looks very bright indeed.

Extensive referencing to recent research on microbe–electrode interactions can be found at the following web sites:

<http://www.microbialfuelcell.org>

<http://www.geobacter.org>

Listening to microbial conversations

Michael J. McInerney, Department of Botany and Microbiology, University of Oklahoma, 770 Van Vleet Oval, Norman, OK 73019, USA.

The microbial world contains a vast and untapped reservoir of genetic diversity that could be used for the production of novel, biologically active molecules or to develop new strategies to manipulate the activities of microbial consortia. However, we need to understand how microbial species interact and communicate with each other in order to manipulate their interactions. With pyrosequencing and other technological breakthroughs, we are beginning to understand ‘who is there’ and ‘what they are capable of doing’. What we need to do is to get

better at understanding ‘what they are doing’ to exploit fully the diversity of the microbial world. We have made great strides in computational approaches that allow us to assign putative functions to many of the genes present in microbial genomes; but, even with these tools, many coding regions lack functional assignments. Many genomes contain ‘cryptic’ or ‘orphan’ gene clusters with the potential to produce novel and structurally complex chemicals (Challis, 2008; Fischbach *et al.*, 2008). These chemicals do not have ‘housekeeping’ functions, but probably function as signals mediating interactions among microorganisms and between microorganisms and eukaryotes (Straight *et al.*, 2006; Dietrich *et al.*, 2008; Fischbach *et al.*, 2008). These studies suggest that microbes are carrying on a conversation with each other. We must listen to and translate this conversation to understand how microbial species interact with each other. Once we understand what microbes are saying and why, then we can manipulate the conversations.

We should not be surprised that microbes converse because we know that microbial species work in teams or guilds. These interspecies interactions must be coordinated, which means that there must be specific signals. In the future, we will have a variety of high-throughput tools to identify how microbial populations respond to each other and what molecules are used. Such approaches may be analogous to microarray technologies such as GeoChip (He *et al.*, 2007). Computational approaches will be available to identify the key regulatory components that receive, translate and transmit the signal to action. As the regulatory networks are defined, we will be able to identify the input chemical stimulus, its receptor and how the signal is transmitted within the cell. Bioengineers can then construct multi-component systems from libraries of standard interchangeable parts engineered from the components identified during the ecological screening process. Professor Endy and his colleagues (Canton *et al.*, 2008) have developed BioBrick (<http://partsregistry.org/>), a standard biological parts inventory, which includes protein coding sequences and regulatory elements for gene expression and signalling and have defined quantitative measures of performance that will allow bioengineers to use these parts reliably. Future efforts will certainly expand on the parts and chassis (organisms) available for manipulation. By understanding how biosynthetic genes change, move about and recombine, we can understand the processes that generate small-molecule diversity.

Once we understand the microbial conversations, we will have the ability to manipulate the response of a specific microbe or of microbial communities. We will be able to identify signals to turn on gene systems to produce new biologically active molecules that could be used as antibiotics or anticancer drugs. Additionally, we may identify