Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Microbiology

Microbial goods from single cells and metagenomes

Editorial overview

Martin Keller and Juan L Ramos

Current Opinion in Microbiology 2008, **11**:195–197

Available online 10th June 2008

1369-5274/$ – see front matter

DOI 10.1016/j.mib.2008.05.003

Martin Keller

Oak Ridge National Laboratory, One Bethel Valley Road, Oak Ridge, TN 37831, USA
e-mail kellerm@ornl.gov

Martin Keller is currently the Director of the Biosciences Division and the Director of the BioEnergy Science Center. His research interests are primarily single-cell microbiology and biofuels research with focus on microbial conversion of lignocellulosic plant material.

Juan L Ramos

EEZ-CSIC, C/ Prof. Albareda 1, E-18008 Granada, Spain

Juan-Luis Ramos is a full-time professor of the CSIC and a member of the EC-US Task Force on Environmental Biotechnology. His main research interest is biodegradation from a wide perspective ranging from aspects of gene regulation to microbial ecology.

All current environmental genomics studies are originated from Norm Pace's cultivation-independent survey approach to study natural microbial populations followed by the extension to use large genome fragment analyses for the characterization of uncultivated microbes. During the past couple of years, we have seen an explosion in total genome and metagenomic projects on the basis of a shotgun-sequencing approach. This has furthered our understanding of the metabolic potential of microorganisms occupying various environmental niches and led to the discovery of many novel biotechnology-related products. In addition, the collection of 'post-environmental genomic' tools is increasing with microarray, proteomic, and metabolomic applications building on the success of environmental genomic discoveries. At the same time we realize, originating from this substantial increased environmental sequencing capacity, that the microbial diversity we find in almost all environments is much larger than ever anticipated. The presence of this tremendous diversity in combination with the finding of significant lateral gene transfer within these environments challenges the conventional understanding and definition of a microbial species and the evolution of microbes. Questions arise if genomes are really discrete or change and adapt to the need and pressure induced through the specific environment, raising the question how significant it is to study single microbial strains in isolation and outside the context of the specific microbial environment. Recent studies have also shown that vast numbers of diverse genotypes can exist at different geographical locations within a single species. Current shotgun sequencing and *in silico* assembly algorithms are challenged by this inherent intraspecies genetic complexity. The fact that we are trying to understand complex microbial environments and the function and contribution of certain microbial ecotypes, makes the binning of sequences an important and challenging task. Single-cell genomics, the total genome sequence from a single or a very few cells, is becoming reality. The ability to identify, separate, and sequence an entire genome from a single bacterial cell might overcome some limitations of current metagenomic approaches and enables genomic analyses such as the characterization of genetic heterogeneity in a population of cells. These cell-specific targeting approaches move toward the understanding of microbial environments from the bottom up and could complement more global bulk-scale measurements. Bulk-scale measurements made on heterogeneous populations, or complete microbial environments, show only averaged values for the population or the environment. The contribution of single microbes will be normalized and only the average population trait will be visible. Targeted metagenomics through whole genome amplification from a single cell will complement environmental metagenomic data and will allow assigning individual genes to the corresponding microorganisms.

Ishoey *et al.* in this issue of Current Opinion in Microbiology elegantly summarize the advances in single-cell bacterium DNA sequencing on the

basis of the so-called 'Multiple Displacement' Amplification' method, which as mentioned above, will speed up research in a large series of uncharacterized environmental organisms. These authors mention how the combination of up-to-date organic chemistry genomics may revolutionize the field of biotechnology. In short, the target is to identify chemicals synthesized by environmental microorganisms through the use of mass spectrometry. Once these chemicals are identified, DNA sequencing of single cells will allow us to identify the gene(s) involved in the biosynthesis of these chemicals. It then follows that single-cell DNA sequencing will not only provide key information for those microbes that we still do not know how to grow in the laboratory but also will allow us to rescue gene clusters of relevance for the ever-increasing white biotechnology industry.

Although great efforts have been devoted to sequence the unsequenced environmental genomes, Czeschowska *et al.* in this issue dig into important questions that can be now addressed at the level of the single cell using evolved flow cytometry tools. These questions range from the study of the physiology of single cells under relevant environmental conditions to the analysis of the always controversial viable versus nonviable cell states, but even more with the current Flow Cytometry techniques, the transition phases can be approached. The key element here is the use of specific dyes incorporated by viable cells (SYTO whose fluorescence is green) by and nonviable cells (PI whose fluorescence is red). This 'colored' approach is complemented by a wealth of techniques that allow the measurements of other biological parameters (respiration rates, nucleic acid content, etc.).

New sequencing technologies realizing much greater sequencing coverage with a significantly reduced cost will be essential to single microbial cell genomics. Lower sequencing costs in combination with targeted metagenomic technologies, such as single-cell genomics, will further revolutionize microbial ecology. In the near future, sequencing costs will go down even further with extended sequencing reads making sequencing of complex microbial environments and broad single-cell genomic studies cheaper and more feasible.

Cheap sequencing techniques will also be essential to determine the dimension of the diversity found in microbial eukaryotes and viruses within the surface oceans. Massana and Pedros-Alio report in this issue that the recognition of the importance of minute eukaryotes as primary producers, bacterial grazers, and parasites paralleled the interest of identifying the species of these probably very diverse assemblages. Allen and Wilson report that viruses, the simplest life form, are extremely abundant in ocean waters and play a crucial role in shaping all kinds of life processes in our planet. Massana and Pedros-Alio show how the analysis of 18S rDNA sequences from various marine environments demonstrated that diversity of minute eukaryotes is extensive since environmental sequences are in almost all cases very different compared to the sequences from cultured strains and an increase in marine eukaryotic diversity occurs at almost all possible phylogenetic scales.

In addition to the finding of increased diversity within marine microbial eukaryotes, environmental shotgun sequencing has uncovered tremendous diversity within planktonic microbial communities. Egan *et al.* report that similar studies are underway to explore surface-associated communities on eukaryotic hosts. The mining of these environments will lead to the discovery of novel enzymes and pathways. Marine sessile eukaryotic surfaces harbor distinct, diverse, but poorly explored microbial communities. These communities show complex interactions between the microorganisms and their hosts, leading to this phylogenetic and functional diversity. The application of novel culturing methods in conjunction with culture-independent methods will enable the exploration of these communities and the search for novel bioactives and enzymes.

Allen and Wilson describe that in a single milliliter of ocean water there are as many as 10^6 viruses and therefore their abundance and diversity seems to be almost infinite considering the volume of water covering the entire planet. The exploration of the world of viruses at the single unit requires extra efforts because genetic information can come in different formats (dsDNA, ssDNA, and RNA). Future research in the field will require considerably new statistical approaches to learn about the function of the plethora of genes of unknown function on the genomes of viruses. What can already be extracted is that viruses can shape ecological niches not only because they infect, proliferate, and kill cells but also because they bear genes that can influence host metabolism, such as sphingolipid biosynthesis, phosphorous homeostasis, photosynthesis, or carbon metabolism.

Significantly increased sequencing capacities, leading to the discovery of novel diversity in almost all investigated environments, is also the foundation for functional genomic studies. Proteomics and transcriptomics have made significant contributions to the field of functional genomics. However, an understanding of the genome, transcriptome, and proteome are not enough to fully characterize cellular function. New mass spectrometers with significantly improved sensitivity and mass accuracy drastically advanced the field of metabolomics. Garcia *et al.* describe the technical advances in chromatographic separation techniques to resolve compounds of different physiochemical properties. Given the complexity of microbial metabolic extracts, a chromatographic separation is required to reduce isobaric interferences and ion suppression. The most comprehensive metabolite cover-

age was therefore achieved by combining multiple and overlapping separations that compliment each other in their ability to resolve compounds of differing physiochemical properties.

Beloqui *et al.* go deep into the concept of single-cell factories as a means to exploit the hidden catalytic potential in metagenomes. Robotized mining strategies have led to a limited number of new functions to synthesize novel products, but the potential is there and intelligent and innovative strategies will arise to produce better biofuels than the most common bioethanol, classical enzymes with side activities will become useful to produce new chiral cores for the subsequent synthesis of novel antimicrobial, anticancer chemicals or new pathways for the removal of pollutants from the environment. To conclude, this issue of Current Opinion in Microbiology is a compendium of reviews that explore the world of single cells; how to sequence a cell genome or a whole population genome (metagenome) and how this information can be translated into useful physiological information and even open industrial perspectives.