TrendsTalk

How Has Microbiology Changed over the Past 25 Years?

In honor of our 25th anniversary, we wanted to take a look back over these past 25 years. Here, we asked members of our editorial board to reflect on the changes that have occurred within the field of microbiology during this time.

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By our understanding of their fundamental nature

I go back twice as far as that, now, having been already 11 when Watson and Crick published their epochal note in *Nature*. So for me, it's barely credible that we can sequence DNA at any speed and cost. That we'd now be assembling complete genomes from single cells or complex environmental samples, and have more than 100 000 sequences available online – that there even is an 'online' – is all like science fiction. But parallel to this technology-driven and astounding advancement there has been an interesting coming-full-circle aspect to our thinking about what microbes really are, their 'ontology', mapped out in a 2013 paper, Microbial neopleomorphism (*Biology and Philosophy* 28, 351–378). In this widely uncited review I drew parallels between the belief in bacterial pleomorphism – that bacteria had no fixed shape or reproductive mode and might adopt multiple physiologies – popular at the end of the 19th century and the beginning of the 20th century, with our current understanding of genomic plasticity. Pleomorphism gave way to monomorphism (and a notion of discrete species lineages) and it was on this and the pure culture paradigm that bacterial genetics (indeed that bacteria have genetics) was founded. Not so indirectly, bacterial molecular biology informed our understanding of our own genome.

The full circle here is that many of us now question the fixity or easy circumscription of bacterial species, given pangenomics and the depth and breadth of lateral gene transfer. And we have come again to thinking of microbes functioning as collectives, among which must be communities of tiny bacteria and archaea that have to feed off each other's gene products to make a living, a sort of terminal Black Queen scenario that encourages some among us to go all Gaia when contemplating the collective metabolism of our planet. 'Neopleomorphism' captures much of this way of thinking and has a nice ring. How true is it? is the next big question.

With major advances about how microbes function and how they impact on human health and disease

As someone who has lived microbiology for the past 40 years, there have been profound changes in the field. It has gone from a field of focusing our knowledge on a few select microbes, some beneficial and some pathogenic, to having a much greater understanding of an incredible number of microbes. Development of new and powerful technologies has driven much of this, as well as the realization that microbes not only cause disease (we have known this for 125 years, thanks to Robert Koch and Louis Pasteur), but that they play a profound role in our health and how the world functions in general. In 1993 there were primitive cloning and sequencing tools available (they certainly didn't seem primitive at the time). Now one can easily, cheaply, and rapidly sequence entire genomes, or mutate pretty much any gene with ease. We have gone from studying microbes as isolated cultures to following them as they live in communities in biofilms, tissue culture cells, or even artificial intestines. Not so long ago, it was said that most of the microbes in the gut couldn't be cultured; now the opposite is true. We can determine expression of all the genes within a microbe, identify many of the complex molecules they make, and have vast databanks full of

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genomic sequences to play with. Our understanding of how pathogens cause disease has moved from a rudimentary understanding of virulence factors (mainly toxins) to very sophisticated understanding of secretion systems that inject bacterial effectors into host cells and reprogram those cells. We have moved from tissue culture models to relevant animal infection models, and are now able to seamlessly incorporate the immune system, host microbiota, and other factors into a holistic understanding of infectious disease processes.

In the past decade, there has been an explosion of information about the microbiome, and it has surpassed interest in microbial pathogenesis, and become one of, if not the, most exciting area in biological sciences. Realizing that the 10–100 trillion microbes living in and on each of us are intricately tied to our health and disease has been a profound realization. From the second we are born into a bath of microbes, these organisms play critical roles in developing our immune system, gut, and even our brain. Nine out of the ten top reasons we die now have microbial links, and *Clostridium difficile* infections can be cured by fecal transplants! If one goes to an immunology, neurology, cancer, or even cardiovascular disease meeting, there are bound to be microbiology talks.

The next 25 years will be an incredibly exciting time for microbiology, as we begin to seriously understand microbial communities, and to exploit much of this information, be it in environmental microbiology or in clinical medicine. One can't predict the future, but what is obvious is that microbiology is undergoing a renaissance and will be a fascinating field to follow for the next 25 years.

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Technology has made the largest impact over the past two decades

Our ability to rapidly and cheaply sequence a whole genome, as well as determine the activity of genes in a given biological situation through RNAseq, has opened our eyes to the inner workings and complexity of bacteria and viruses. It initiated the microbiome field where the intricacy and diversity of microorganisms inhabiting a particular niche within the human body can now be more fully appreciated. It opened our eyes to the importance of the accessory genome (in particular bacteriophage) in the day-to-day survival of bacteria, both in the environment and in the human body during health and disease. Technology has also revealed the ways in which phages manipulate the bacterial genome for its own survival and the survival of its host, pointing more and more that phages control the biosphere.

A glaring deficiency in this technological revolution is the publication process. Why accelerate the generation of data by months to years when it takes many months to get that information to the scientific community? Thus, to fully exploit these technological advances, the publication process must be reinvented to a system that is rapid, fair, and accurate.

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Our microbiome as a major player in health and disease

Some 150 years ago modern medical microbiology emerged through the ground-breaking work of Louis Pasteur and Robert Koch. This scientific breakthrough culminated in Koch's postulates, which can be simplistically reduced to one sentence: One specific pathogen causes one specific disease. To Pasteur and Koch the human host lacked appropriate defense mechanisms. But soon thereafter, their disciples, Emil Behring, Paul Ehrlich, and Elias Metchnikoff, developed the concept of immunity as a measure to defend the host against invaders. This concept of infectious diseases as an outcome of the cross-talk between pathogen and host allowed the rational development of effective intervention measures, including antibiotics, diagnostics, and vaccines, which led to a significant reduction in communicable diseases.

Over the past 25 years, a new concept arose. Although it was known for a long time that humans are colonized by harmless microbes, technological advances allowed investigations into the microbiome which provided an important complementary concept to our understanding of microbes' impact on health and disease. Each of us is colonized by an enormous number of microbes. With 10^{12} microbes from some 500 different species on a single host, the abundance of microbial cells roughly equals that of human cells in our body. The qualitative and quantitative characterization of our microbiome was followed by epidemiologic studies and mechanistic animal experiments aimed at better understanding the role of the microbiome in communicable and noncommunicable diseases. It thus became clear that a well-balanced ecosystem not only shapes numerous physiological activities but is also critical for our wellbeing.

Once the microbial ecosystem is out of balance, different types of disease develop. Thus, following depletion of the microbiome by antibiotic treatment, *Clostridium difficile* can expand in the gut and cause severe gastroenteritis and diarrhea. The microbiome also contributes to obesity which is transferable by microbiota from obese animals. Moreover, for example, through the production of cancerogenic compounds, the microbiome is involved in colorectal cancer. At the next level, a disbalanced microbiome impacts on metabolic diseases and bowel diseases primarily through inflammatory responses. The microbiome also affects distant sites of the body, contributing to cardiovascular, liver, and skin diseases, and the brain-gut axis relates the microbiome to neurodegenerative diseases as well as depression.

Obviously, the composition of the microbiome strongly depends on the type of nutrients it is offered. Hence, prebiotic nutrients and probiotic microbes provide an opportunity to actively influence the composition of microbiota for the better, as had already been foreseen by Metchnikoff some 100 years ago. The elucidation of the microbiome as an ecosystem which is determined by the quantitative composition of different species extends the canonical concept of the causative role of pathogens in communicable diseases to the impact of microbial ecosystems on noncommunicable diseases. I have no doubt that further insights into this exciting cross-talk will pave the way for novel intervention measures.

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Pathogenic bacteria as a new toolbox

Over the past 25 years, the field of bacterial pathogenicity is one of the most advanced areas in microbiology. The bacterial genetic factors called 'effectors', which are secreted via the type III and type IV secretion machineries of Gram-negative bacteria, have been identified as virulence-associated proteins, but their exact roles in infection remained poorly understood. Fortunately, thanks to dramatic developments in innate immunology, cell biology, structural biology, and bioinformatics, we were eventually able to characterize many effectors as key players in bacterial infection. To our surprise, these effectors mostly mimic the functions of mammalian enzymes in order to subvert and modulate host defense barriers, cell regulatory systems, and innate and acquired immune systems for their own benefit.

Our toolbox for studying such highly pathogenic bacteria is currently being enriched by tools such as omics analysis, genomic editing, genetically modified animals, single-molecule imaging, and single-cell analysis, which have greatly improved our understanding of how bacterial pathogens target and create niches within host tissues. Meanwhile, the world is facing a growing threat of emerging and re-emerging infectious diseases and multidrug-resistant bacteria. Therefore, over the next 25 years, the study of bacterial pathogenicity and host defense responses using the new toolbox will lead to the development of novel types of vaccines and drugs aimed at controlling infections.

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Imaging – seeing is believing – and appreciating!

Microbiology is by definition the science of organisms too small to be observed by the naked eye, and so we have always relied upon microscopy to observe and understand microbes. Huge progress has been made since the first light microscopic observations of bacteria and protozoa made by van Leeuwenhoek in the 17th century. Although viruses were discovered in the late 19th century using filters too small for bacteria to pass, it was the advent of electron microscopy in the 20th century that allowed their visualization.

Electron microscopy (EM) has been central to viral identification and classification, and has seen a quantum leap in the form of cryo-EM. The 2017 Nobel Prize in Chemistry was awarded for work on cryo-EM, and this technique is leading the way in describing the organization of macromolecules, many microbial, at near-atomic resolution. Cryo-EM has advantages over crystallography, including absence of the requirement for a crystal lattice, the ability to extract structural information from complexes *in situ*, such as embedded in membranes, and a predilection for large, often hard-to-crystallize molecular complexes. Cryo-EM tomography and single-particle analysis have already made essential contributions in many areas of microbiology, including high-resolution structures of bacterial ribosomes, chemoreceptors, and secretion complexes; non-enveloped and enveloped viral architecture; and parasite host cell invasion machines and drug targets. A specific example in my own field has been the amazing breakthrough in understanding the structure and function of the HIV-1 envelope glycoproteins, and its implications for antibody-based vaccine design.

Over the same period, light microscopy technologies have been developed that break the diffraction limit of light to yield 'super-resolution'. This again resulted in a Nobel Prize in Chemistry in 2014, and has allowed us to overcome the challenge of imaging both cells (about 10 μm) and microbes (from ~ 20 nm for some viruses to ~ 50 μm for

some protozoa) using light microscopes. Super-resolution microscopy has revealed exciting new details such as virus–receptor interactions at cell surfaces and changes in cellular organization and architecture during pathogen invasion. Although with lower resolution, two- and multi-photon fluorescence intravital microscopy has revolutionized our understanding of events taking place *in vivo*, since this technology allows light to penetrate tissue and report back on dynamic events, without substantially perturbing the system. Using this approach we have been able to visualize the behavior of cells and microbes within living tissues, often within the intact host, revolutionizing our understanding of microbial pathogenesis and immune responses to pathogens.

What does the future hold for imaging in microbiology? Cryo-EM will yield higher resolution structures of more and more complex microbial machines, facilitating their targeting with drugs and vaccines; intravital microscopy will allow us to better understand host–microbe interactions under the most physiological conditions; and super-resolution microscopy will fill in the gaps between these technologies to allow detailed descriptions of host–pathogen interactions. The future for microbiology looks colorful, detailed, and bright!

<https://doi.org/10.1016/j.tim.2018.02.002>

Special Issue: Broad Concepts in Microbiology

Spotlight

Towards a Natural History of Soil Bacterial Communities

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Despite the enormous diversity of bacteria, a recent study reveals that soils are globally dominated by a small list of taxa. Characterizing the traits of these bacteria offers the potential for predicting functional differences among soil communities.

It is difficult to overstate how far our understanding of microbial biogeography has advanced over the past 25 years. Nowhere are these advances clearer than for soil bacteria. It is now known that soil bacterial communities display many of the same biogeographic patterns observed in plants and animals, including taxa-area curves, distinct biome communities, and

predictable variation with abiotic parameters [1,2].

Still, the diversity of soil bacteria is overwhelming. There are billions of bacterial cells, and perhaps a million species, in just a handful of soil [3,4]. No wonder most studies give few details about the particular bacteria present in their soil samples. It is not difficult to find studies that report that bacterial composition differs among samples without naming any specific bacterial taxa [operational taxonomic units (OTUs) or more recently, single nucleotide variants]. While most studies might give a cursory mention of the most abundant phyla or orders, finer scale genus- or even family-level information is often considered overly descriptive.

This custom is in stark contrast to studies of larger organisms, which typically describe the most common species within a study site. This sets the scenery of the study as ecologists can relate this information to their natural history understanding of similar ecosystems. A list of the abundant species paints a picture of the community and

the abiotic history of the ecosystem. The dominant species in a plant community reveal whether the study takes place in a grassland or a forest, whether that forest is evergreen or deciduous, and if it is old-growth or undergoing succession.

A recent study by Delgado-Baquerizo *et al.* [5] suggests that, despite the incredible diversity of soil bacteria, soil microbiologists might also be able to develop a sense of the natural history of their communities. The authors first identify the most abundant and widespread bacterial taxa (defined by $\geq 97\%$ similarity of 16S rDNA sequences) within a suite of soil samples from around the globe. These dominant taxa make up only 2% of soil diversity. More surprisingly, however, this small fraction of total diversity (~ 500 taxa) accounts for a large fraction ($\sim 40\%$) of all sequences from soil samples around the world. This result means that, in any soil sample, a selection of these taxa will be present and highly abundant. Despite the vast amount of undescribed bacterial diversity in soils, much of the bacterial biomass could be described by a reasonably short list of taxa.