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The National Microbiome Initiative

Tom Schmidt

We squeezed together under a small roof near the guard station just outside of the White House to avoid the drenching rain. There was excitement in the air as we waited for the launch of The National Microbiome Initiative (https://www.whitehouse.gov/blog/2016/05/13/announcing-national-microbiome-initiative). I thought about the cloud of microbes surrounding each of us and how with something as simple as a breath or a handshake, our microbiomes were intermingling. Nature might have selected for such exchanges as a means to maintain the diversity of our microbiomes, but a microbial interchange with people gathered from around the country was definitely not in our evolutionary past.

Fortunately, we didn’t have to wait for long before we were out of the rain and Jo Handelsman, associate director of the White House Office of Science and Technology Policy, welcomed a crowd of approximately 200 to the event. She had worked diligently to bring national attention to microbiomes and was now addressing a room full of people, many of whom, like her, had devoted a majority of their lives to making discoveries about the world of microbes. Through the National Microbiome Initiative, the Obama administration was bringing considerable visibility and resources to the study of microbiomes much as it had with other emerging scientific fields including the Brain Initiative (2013), the Climate Education and Literacy Initiative (2014) and the Precision Medicine Initiative (2015). It is worth noting that microbes have dramatic impacts on each of those endeavors as well.

The National Microbiome Initiative recognizes the importance of microbiomes—communities of microorganisms and their habitats—not only in and on humans, but also those associated with plants, terrestrial environments, the oceans, and the atmosphere. The initiative also offers the public a reason to care: “Microbiomes maintain healthy function of these diverse ecosystems, influencing human health, climate change, food security, and other factors.”

One urgent challenge in studying almost any of these microbiomes is the annotation of nucleic acid sequences. In this issue of Microbe, Valérie de Crecy-Lagard suggests that as many as 2/3 of genes in public databases are either poorly or incorrectly annotated! This is certainly a humbling estimate for those of us who rely on annotations to build metabolic models of microbes and microbial communities. It would be productive to direct some of the funding from the National Microbiome Initiative to de Crecy-Lagard’s recommendation to “…build international collaborations for improving and curating gene annotation in central repositories.”

Funding opportunities for the National Microbiome Initiative come from numerous federal and private agencies, many of who were on hand to announce their commitment to microbiome research. Additionally, an innovative program to support the development of next generation scientific tools for investigating life on a microbial scale was announced by the Kavli Foundation at an evening reception hosted by ASM (http://www.kavlifoundation.org/kavlifoundation/kavli-news/american-society-microbiology-launches-kavli-microbiome-ideas-challenge-partnership-two#.V2pytVetXsE). As we begin to consider strategies to manage microbiomes for our health and the health of the planet, let’s take advantage of the national attention and funding to stimulate fundamental, creative, and collaborative research. It can only help to integrate discoveries, technologies, and strategies for studying microbiomes in diverse environments because there are so many challenges to understanding the complex world of microbes.

Tom Schmidt, Professor, Center for Microbial Systems, University of Michigan, Ann Arbor, is a member of the Microbe Editorial Board.
Journal Impact Factors: Changing the Weather

The Journal Impact Factor is not making a positive contribution to science, and ASM will no longer support it for its journals

Stefano Bertuzzi, Lynn W. Enquist, Joseph Campos, James Tiedje, Timothy J. Donohue, and Susan E. Sharp

The Journal Impact Factor (JIF) is like the weather: everyone talks about it, everyone complains about it, and everyone feels incapable of changing it. Indeed, the scientific community has been held hostage of this measure of impact for a long time, which erroneously became the one and only simple metric to evaluate the impact of a single publication, the prestige of a journal, or the relevance of an individual scientist.

This is the result of a big misunderstanding of the JIF and not recognizing its significant shortcomings. Scientists, scholarly journals, and everyone else who has a stake in proving impact started chasing the JIF and touted the “high impact factor,” often forgetting to read the paper, or critically evaluating what was said in a paper, focusing instead on the JIF of where the paper was published.

When Eugene Garfield, together with Irving Sher, thought of the JIF in the early 1960s, it was simply to normalize citations so that journals could be compared regardless of their size. Their original intent was to measure the frequency with which an “average article” in a journal was cited so that smaller source journals, in addition to large, frequently cited journals, would be included in their Science Citation Index database (Garfield E. 2006. The history and meaning of the Journal Impact Factor. JAMA 295:90-93; doi:10.1001/jama.295.1.90). Of course, they understood that there are no “average” articles, just as there are no “average” scientific fields, but the JIF took on a life of its own and very quickly became a measure of scientific relevance in evaluating the science.

Just like the weather, there have been many rumblings about the JIF, but unlike the weather, the daily misuse of the JIF culture to assess scientific research can be changed. In 2013, a group of scientists and publishers released the San Francisco Declaration on Research Assessment (DORA; http://www.ascb.org/files/SFDeclaration FINAL.pdf). The goal was to come together to emphasize the need for scientists, funders, and academic institutions to evaluate published research on its own merits, not on the flawed misuse of the journal impact factor. To date, DORA has been signed by more than 12,000 individual scientists, editors, and funders and over 700 organizations, quickly becoming known all over the world, publicly initiating debate on the misuse of the JIF.

Recognizing the problems of the misuse of the JIF, the ASM Journals Program signed and supported DORA from the early days, but now ASM goes further. During the first ASM Microbe Meeting, held in Boston in June 2016, ASM discussed the misuse of the JIF, and the ASM leadership decided that ASM as an organization—not just its journals operations—will immediately:

1. Sign DORA
2. Remove from the website the JIF for all of its journals
3. Cease using the JIF in advertising or marketing to potential authors and readers

The Society is proud of the reputation for quality and service to the field of its journals, and is hopeful that authors and readers, whether ASM members or not, will continue to recognize their value to the advancement of the microbial sciences.

In conjunction with this announcement, ASM has published an editorial in mBio on the topic (mbio.asm.org).

Stefano Bertuzzi, Ph.D., MPH, is the CEO of ASM; Lynn W. Enquist, ASM Past-President, Princeton University, Princeton, N.J.; Joseph W. Campos, ASM Past-Secretary, Children’s National Medical Center, Washington, DC; James Tiedje, ASM Treasurer, Michigan State University, East Lansing; Timothy J. Donohue, ASM Secretary, University of Wisconsin, Madison; and Susan E. Sharp, ASM President, Kaiser Permanente Northwest, Portland, OR.
Slow Movement on Antibiotic Resistance

Could inspiration to trigger real action on the global medical problems caused by bacterial drug resistance come from a musical source?

Bernard Dixon

I had just started telling a friend how regrettable it was that many doctors prescribe antibiotics for trivial infections, even for minor troubles that might be virus infections or not even infections at all, when he interrupted—pleading agreement with every word. “You’re quite right,” he insisted, “I would never dream of troubling my doctor for trivial complaints. I go to the Internet and find the antibiotics I want there. Years ago, every time I went to a country where the stuff was available across the counter, I used to stock up.”

Whenever I have this sort of experience, or read of the latest horrendous problems created by antibiotic-resistant bacteria around the world, I think above all of one man—E. S. (Andy) Anderson, head of the Enteric Reference Laboratory in London in the 1970s, who did more than anyone to warn of encroaching disasters unless action were taken to stem the tide of resistance. Writing in the *Journal of Hygiene* (74:289, 1975) and elsewhere, he warned how vital it was to restrict any increase in the population of bacteria invulnerable to antimicrobial drugs. This was a tough message, on a planet where astronomical quantities of antimicrobials were deployed without reason or responsibility—by people buying them across the counter; by doctors satisfying patients’ expectations or being thoughtless and sloppy; and by farmers, veterinary surgeons, and feedstuff manufacturers, all of whom connived at the misuse of these potent agents in animal husbandry.

Again and again, Andy pointed out both the immediate and long-term hazards caused by invulnerable pseudomonads that were so feared in burns units, the ampicillin-resistant *Haemophilus influenzae* strains that were beginning to cause untreatable meningitis among children in the United States, and the chloramphenicol-resistant *Salmonella typhi* that had already infected some 100,000 patients in Mexico and killed 14,000 of them. These were not nuisance species of concern only to one country, Andy observed. They were part of the world population of bacteria, and action to prevent their emergence or limit their spread needed to be taken on a correspondingly international basis.

Much of Andy’s paper described laboratory and epidemiological work with chloramphenicol-resistant isolates of typhoid bacilli from Mexico, India, Thailand, and Vietnam—countries without any restrictions on the sale of antibiotics, and where indiscriminate drug usage led to a high incidence of resistance factors in the nonpathogenic enterobacteria of humans and animals. Moreover, the Mexican epidemic in particular was not limited to the indigenous population. British, Swiss, and many American visitors also became infected. The R factor responsible conferred not only an unusually high level of resistance to chloramphenicol but also insensitivity to streptomycin, sulfonamides and tetracyclines. A closely related R factor had been isolated from cultures of *S. typhimurium* from Portugal, Belgium, Canada, and Israel. Other closely related problems included epidemic drug-resistant dysentery caused by *Shigella disenteriae* in Central America and intractable, virulent outbreaks of infection with multiply resistant salmonellae in pediatric units in South America.

As Anderson pointed out, such developments were predictable and provided yet another warning that rationalization and reduction of the use of antibiotics and other antibacterial drugs were necessary not only in developing countries but throughout the world. “The time has clearly come,” he concluded, “when international cooperation at legislative and professional levels is needed to attempt to reverse the change in the ecology of the enterobacteria and other organisms that has resulted from the indiscriminate use of antibacterial drugs.”

Although his voice was arguably the strongest
to call for real, global action on drug resistance, Andy was not alone. And in the intervening years, many other individuals and committees have taken up the cause—most recently Britain’s senior medical officer, Dame Sally Davies, who recently argued that “antimicrobial resistance risks a health catastrophe to rank with terrorism and climate change.” Yet the situation remains grim, leading to repeated warnings that we are in danger of returning to the dark ages when bacterial infections were literally untreatable.

And of course, microbial evolution has continued apace—based not only on the mutation and selection that would have been familiar to Charles Darwin but also on the transferable resistance that makes matters (from a human perspective) considerably worse. One of the latest, alarming developments is the emergence of the first plasmid-mediated polymyxin resistance mechanism, MCR-1, in Enterobacteriaceae. Writing in *The Lancet Infectious Diseases* (16:161, 2016), Yi-Yun Liu and other investigators in China point out that this heralds the breach of the last group of antibiotics by plasmid-mediated resistance. “Although currently confined to China, MCR-1 is likely to emulate other global resistance mechanisms such as NDM-1,” they write. “Our findings emphasise the urgent need for coordinated global action in the fight against pan-resistant Gram-negative bacteria.”

How grimly familiar those words would sound to E. S. Anderson, more than half a century after he first enunciated similar sentiments. Andy did at least have the satisfaction of stirring up a political storm on the subject in the UK, supported by several influential figures in the media. Eventually, this triggered off a government inquiry into the (mis)use of antimicrobials in animal husbandry. Despite stiff opposition from some sectors of the pharmaceutical industry, that committee’s deliberations and recommendations did lead to a total ban on the inclusion of penicillins and cephalosporins in feedstuffs for pigs and poultry, to promote their growth.

Aside from real but limited initiatives of this sort, however, the general picture remains bleak. What on earth can be done now, after decades of a worsening scenario and countless research papers, reviews, newspaper articles, warnings, Web activity, committees of inquiry, and inaction by the individuals and bodies that could actually help to alter the situation?

Even last year’s call by the WHO’s annual meeting in Geneva was deeply unimpressive. True, the representatives of 194 countries approved a new global plan with five main objectives—to improve awareness and understanding of resistance, strengthen surveillance and research, reduce the incidence of infection, optimize the use of antimicrobial drugs, and ensure sustainable investment in the countering of resistance. But these seem little more than worthy, familiar words. And the agreed target of 2017 for every country to have in place a system to monitor drug resistance is pathetic.

Perhaps the requisite inspiration to encourage real action may come not from science alone but from music. The very idea may sound crazy at first, yet I do discern possible help from that quarter. Andy Anderson’s son, Julian, is a composer—currently a professor at the Guildhall School of Music and Drama in London and before that professor of music at Harvard University. Moreover, Julian has written a piece of music, *Transferable Resistance*, which portrays the very phenomenon his father spent a lifetime studying. Commissioned by the Royal Society in London, it is dedicated to the memories of Andy and of two other distinguished microbiologists, Max Delbrück and Bill Hayes. It is played by four groups of brass players located in different parts of the concert hall.

Could it be that someone, somewhere, some time, is so stirred by Julian’s expression of the molecular genetics of antibiotic resistance transfer, and its human consequences, that he or she is emboldened to ensure real progress?
RESEARCH ADVANCES

Bacterial Diversity Is Dominant Feature in New Tree of Life

Carol Potera

A new rendition of the tree of life includes 92 named bacterial phyla, 26 archaeal phyla, and all five of the eukaryotic supergroups. This tree highlights how bacteria dominate all of biology in terms of diversity. Moreover, although many of the most abundant bacteria have never been seen, they were identified using reconstructed genome sequences. “It was a surprise to see the massive scale of diversity in Domain Bacteria, including many lineages that lack isolated representatives,” says study leader Jill Banfield of the University of California, Berkeley. Details appeared 11 April 2016 in *Nature Microbiology* (doi:10.1038/nmicrobiol.2016.48).

The phylogenetic analysis relied on a set of 16S ribosomal protein sequences, as well as 16S rRNA data, according to Banfield. “A particular strength is that these 16S genes occur consistently in close proximity on the genome,” she says. “It’s fundamentally a genome-resolved approach that uses only high-quality, curated genomes, including some complete genomes.” Meanwhile, this new tree shows animals, plants, and fungi crowded onto two small branches, while bacteria occupy the major branch.

The new tree of life “provides a much needed big picture of what’s known about the phylogenetic diversity of bacterial and archaeal genomes,” says Jonathan Eisen at the University of California, Davis, who was not directly involved in this effort. It has implications for better understanding evolution, the functional diversity of microbes, the biases in many methods used to study microbial diversity, the general requirements of living systems, and where to target new studies of microbial diversity, he says. Eisen also commends Banfield for publishing the data and results in an open access manner.

Banfield and her collaborators “show the relevance of genome-resolved approaches to understand microbial life,” says A. Murat Eren at the University of Chicago, who was not involved in the research. This latest depiction of the tree of life, he adds, “likely is not the
final one, if there ever will be one. But it shows how much diversity we’ve missed, despite remarkable technology breakthroughs.”

About half the bacteria arrayed along the dominant branch of the new tree belong to the Candidate Phyla Radiation. Little is known about members of this recently described supergroup, whose members characteristically have small genomes and symbiotic lifestyles. Only one member of the group has been isolated, while one other was recently cocultured. Candidate Phyla Radiation is not a formal name, but rather a working title to describe what is now a prominent part of the tree of life, Banfield notes.

Little is known directly about the metabolic lifestyles of bacterial species belonging to the Candidate Phyla Radiation. Their genomes contain recognizable genes consistent with their having metabolic pathways for replicating DNA and making proteins. However, species falling within this radiation lack genes to complete the citric acid cycle, respiratory chains, and nucleotide synthesizing capabilities. Thus, they likely rely on fermentation for their metabolic energy and derive some of their basic building blocks from other organisms.

Despite these metabolic shortcomings, there is massive diversity within the Candidate Phyla Radiation—a major new finding that highlights this new depiction of the tree of life, Banfield says. Further analysis of their ecology, evolution, and biochemistry, she adds, “may provide clues about the metabolic platform of early life.”

Carol Potera is a freelance writer in Great Falls, Mont.

RESEARCH ADVANCES

New Synthetic Cell Challenges One-Gene, One-Trait Hypothesis

Marcia Stone

“Life is much more like a symphony orchestra than a piccolo player,” J. Marcia Stone

One-Trait Hypothesis Challenges One-Gene, New Synthetic Cell

Challenges One-Gene, New Synthetic Cell

RESEARCH ADVANCES

One-Trait Hypothesis Challenges One-Gene, New Synthetic Cell

Challenges One-Gene, New Synthetic Cell

MINITOPIC

Microbiology Policy Bulletin Board

Recent developments involving microbiology and related science policy matters include:

- The White House, through its Office of Science and Technology Policy, in May launched a National Microbiome Initiative (NBI), which will be comparing microbiomes across different ecosystems, calls for federal agencies to invest more than $121 million in fiscal years 2016 and 2017, in microbiome research. Additional non-federal support for the NBI includes $100 million over four years from the Bill and Melinda Gates Foundation and $10 million over five years from the Juvenile Diabetes Research Foundation.

- At least 30% of antibiotics prescribed in the United States are unnecessary, according to officials from the Centers for Disease Control and Prevention in Atlanta, Ga., and their collaborators from the Pew Charitable Trusts and other public health and medical experts. Details appeared 3 May 2016 in the Journal of the American Medical Association (doi:10.1001/jama.2016.4151).

- Food and Drug Administration (FDA) officials in May recommended that, “unless they lack other treatment options, patients with uncomplicated infections should not receive fluoroquinolones, given the risk for disabling and potentially permanent adverse events.”

- FDA in May revised rules for collecting antibiotic sales data, requiring manufacturers to provide estimates of sales of drugs being used for specific food-producing species—cattle, swine, chickens, and turkeys—in addition to notifying FDA of overall antibiotic sales estimates.


- As part of efforts to eradicate polio, public health officials from 150 countries switched by May from administering a trivalent to a bivalent oral poliovirus vaccine, a change that reflects the loss of wild poliovirus type 2 from circulation—ending a need to vaccinate against it. Removing it from the vaccine eliminates the risk of its reappearing from mutations within the vaccine mix. Meanwhile, in 2015, 74 wild poliovirus (WPV) cases were reported in Afghanistan and Pakistan, a decrease of 79% from the 359 WPV cases reported in 2014 in nine countries, according to the World Health Organization (WHO). As of May, WHO officials note, a mere 12 WPV cases were reported worldwide in 2016.
Craig Venter says about his institute’s new synthetic bacterium. It carries a mere 473 genes, a smaller genome than any autonomously replicating cell ever found in nature. That Venter and his collaborators synthesized a new bacterium and brought it to life in a bacterial corpse is not the biggest part of this story. Perhaps more importantly, their synthetic mini microbe, designated JCVI-syn3.0, contains many quasi-essential genes—genes not absolutely necessary for viability but critical for robust growth. This is not the one-gene, one-trait phenomenon that cell reductionists were wishing for, he asserts. Details appeared 25 March 2016 in Science (doi:10.1126/science.aad6253).

“The idea initially was that a minimal genome would only have essential genes, if you knocked out any gene, it would be dead,” says Clyde Hutchison, Venter collaborator and project leader. “But the finding of all these quasi-essential genes changes our perspective on this.” Cells that grow quickly enough to be useful in a laboratory need the quasi-essential genes that they carry. Thus, Hutchison considers JCVI-syn3.0 a “tradeoff”—a minimalist bacterial cell with a genome small enough to define all the genes in a cell but one having a reasonable growth rate. In fact, with all its quasi-genes intact, JCVI-syn3.0 has a respectable doubling time of about 180 minutes and forms the same sort of colonies as its ancestor JCVI-syn1.0, which itself is a whittled-down version of a wild-type Mycoplasma genitalium.

Mycoplasmas are not small because they are primitive, according to Hutchison. Instead, they derived from cells with several thousand genes apiece, he says. However, because the mammalian host provides them with “a very rich, uniform, and constant environment,” they have lost many genes that they no longer need, moving them towards a minimal genome. “We’re just helping them along to get rid of the genes they don’t need in the laboratory,” he says.

“Evolution loves to ‘streamline’ genomes to save on resources, but it’s unlikely there’s any such thing as a truly ‘minimal’ genome,” says Jeff Morris at the University of Alabama in Birmingham. “The cells eventually get to a point of diminishing returns, where losing more genes starts to cut into growth rate or other aspects of fitness. That’s what Venter’s group appears to have discovered with ‘quasi-essential genes,’ and it’s a great direction to go when pursuing ‘plug-and-play’ synthetic organisms.”

Venter points out that “minimum” is a relative term. For example, a minimal photosynthetic cell will be very different from JCVI-syn3.0. Nonetheless, scientists are “getting close to fully understanding the number of genes and
set of genes required to make a cell grow and divide,” says his collaborator Daniel Gibson. However, adds Gibson, the surprises surrounding the analysis of this most fundamental bacterial cell, where approximately one-third of the genome is not yet understood, raise new concerns about plans to engineer genes in far more complex human cells. “It’s vastly premature” to be editing the human genome with CRISPR/Cas9s,” he insists.

Marcia Stone is a science journalist based in New York City.

RESEARCH ADVANCES

Tunable Laser Monitors Microbes in Packaged Foods, Medical Supplies

Barry E. DiGregorio

Combining a tunable diode laser absorption spectrometer (TDLAS) with wavelength modulation (WM) yields an instrument that can rapidly detect carbon dioxide to monitor microbial growth, including in packaged food products and medical instruments and supplies, according to Jie Shao at Zhejiang Normal University in Jinhua, China, and his collaborators there and at Umeå University in Umeå, Sweden. Details appeared 20 March 2016 in Applied Optics (doi:10.1364/AO.55.002339).

Because TDLAS combines “ease of use and low cost,” it is “particularly suitable” for “assessing levels of carbon dioxide in a given closed compartment,” Shao says. In practical terms, that means this approach can be used for “monitoring microbial growth in industrial settings.” Historically, TDLAS is well known for measuring the concentration of specific gaseous species—including carbon monoxide and dioxide, methane, and water vapor—in various environmental settings, he says. However, it was not previously used for detecting microorganisms.

In proof-of-concept experiments, Shao and his collaborators determined whether the TDLAS instrument could detect carbon dioxide as an indicator of the growth of Staphylococcus aureus bacteria and Candida albicans fungi. In this capacity, WM-TDLAS provides an essentially instant response time, suggesting its applicability for use in food manufacturing settings and also for detecting blood infections in health care settings, according to Shao. The instrument package could cost as little as a few thousand dollars, he points out.

TDLAS “is particularly suitable since it combines . . . ease of use and low cost,” Shao continues. “Existing techniques for assessing microorganism growth do not allow for rapid and accurate assessments; they need often to be carried out at dedicated laborato-

MINITOPIC

Microbiota Studies Involving the Gut or Other Anatomic Sites

Here in brief is another set of findings from recent efforts to understand how microorganisms in the gut or elsewhere in the body interact with or affect the host:

- Mice that received only anti-inflammatory Lactobacillus johnsonii bacteria produce metabolites that prevent cancer and had more efficient fat and oxidative metabolism than did mice that received a mix of inflammatory and anti-inflammatory microbes, according to Robert Schiestl of the University of California, Los Angeles, and his collaborators. Further, animals in the first group formed lymphomas only half as fast as did those in the second group. Details appeared 13 April 2016 in PLoS One (doi.org/10.1371/journal.pone.0151190).
- Bacteria-derived gut metabolites affect myelin content irrespective of the genetic makeup of the mice in which they produce those metabolites, according to Patrizia Casaccia at Mount Sinai School of Medicine in New York, N.Y., and her collaborators. “Our findings will help in the understanding of microbiota in modulating multiple sclerosis,” she says. Details appeared 20 April 2016 in eLife (doi:10.7554/eLife.13442).
- His recently developed, multilayered model distinguishes host-adapted core microbiota from an environmentally modulated flexible microbial pool—noting that gut microbiotas expand current notions on how symbionts shape host evolution, says Michael Shapira of the University of California, Berkeley. Details appeared 30 March 2016 in Trends in Ecology and Evolution (doi:10.1016/j.tree.2016.03.006).
- Based on experiments with mice, it may be possible to target central nervous system abnormalities such as posttraumatic stress disorder by manipulating gut bacterial communities, according to John Bienenstock at McMaster University in Ontario, Canada, and his collaborators. Details appeared February 2016 in the Canadian Journal of Psychiatry (doi:10.1177/0706743716635535).
- Bacteroides species dominate the gut microbiota of infants in Finland and Estonia, where early-onset autoimmune disorders are common, but not among infants in Russia, where such diseases are rare, according to Ramnik Xavier at the Broad Institute of the Massachusetts Institute of Technology and Harvard in Cambridge, Mass., and his collaborators. Further, the lipopolysaccharide of Bacteroides, but not of Escherichia coli, inhibits innate immune signaling and endotoxin tolerance in mice, suggesting that Bacteroides dominance may “preclude aspects of immune education,” they note. Details appeared 5 May 2016 in Cell (doi: 10.1016/j.cell.2016.04.007).
MINITOPIC

Recent Developments in Microbial Diagnosis and Detection

Recent developments in detecting microorganisms include:

- A prototype method for identifying bacterial pathogens, called polarization anisotropy diagnostics (PAD), uses light to detect specific bacterial RNA sequences, and yields results for several dozen bacterial pathogens of humans within a few hours at a cost of about $2 per assay, according to Hakho Lee of Massachusetts General Hospital in Boston, Mass., and his collaborators. Details appeared in 6 May 2016 in Science Advances (doi:10.1126/sciadv.1600300).

- A sensor, based on a polymeric film that specifically captures D-arabitol, a molecular marker for fungi, can rapidly detect fungal pathogens within cerebrospinal fluid, plasma, and urine specimens, according to Włodzimierz Kutnera at the Institute of Physical Chemistry of the Polish Academy of Sciences in Warsaw and his collaborators. Details appeared 15 May 2016 in Biosensors and Bioelectronics (doi:10.1016/j.bios.2015.12.088).

- Ebola virus RNA can be detected with a PCR assay, using a cellphone-sized device that yields results within less than 1 hour, according to Pavel Neužil of Northwestern Polytechnical University of Shaanxi, China, and Brno University of Technology in Brno, Czech Republic, and his collaborators. Details appeared 11 April 2016 in Analytical Chemistry (doi:10.1021/acs.analchem.6b00278).

- In Tanzania, a mobile-phone-based system is being used for rabies surveillance over a large-scale area of about 150,000 km². It is being used to evaluate ongoing rabies control activities and improve their management, according to Katie Hampson from the University of Glasgow, United Kingdom (UK), and her collaborators in the UK and Tanzania. Details appeared 12 April 2016 in PLoS Medicine (doi:10.1371/journal.pmed.1002002).

“Absorption is a problem. You would have to actually test each individual type of container to determine whether you could collect a usable signal. You also would not want anything in the packaging that scatters the infrared light, which could reduce signals to unusable levels.”

Although the cost of using WM-TDLAS-based instruments to monitor microbial growth could be as low as about $0.50 per assay, Lodder suggests that costs would need to be reduced several orders of magnitude further before this approach could be considered “a game changer.”

Barry E. DiGregorio is a freelance writer in Middleport, N.Y.

ZIKA VIRUS

Despite prolonged wrangling over how and how much to fund U.S. research on the Zika virus, the research community is making considerable progress studying this pathogen, quickly confirming its capacity to interfere with fetal development. Beyond careful tracking of this flavivirus and extensive analyses of its structure and genome, recent research efforts focus on understanding its impact on infected individuals, particularly when pregnant, on developing diagnostic tests for the virus, and on vaccine development as well as control of the mosquitoes that spread this virus.

“In less than a year, the status of Zika has changed from a mild medical curiosity to a disease with severe public health implications,” said Director-General Margaret Chan of the World Health Organization (WHO) in Geneva, Switzerland, last March. She also declared the virus “a public health emergency of international concern,” one that requires a “coordinated international response.” As of March, the virus was detected in at least 38 countries and territories. “If this pattern is confirmed beyond Latin America and the Caribbean,” she added, “the world will face a severe public health crisis.” Nonetheless, the emergency committee advising WHO on Zika “found no public health justification for restrictions on travel or trade to prevent the spread of Zika virus.”

Amid that global call for action against Zika, President Obama and members of the U.S. research and public health communities continued to voice frustration through May as they sought additional resources to investigate the Zika virus and slow its spread. One strategy, namely to repurpose resources intended for Ebola virus for use on Zika, remained stalled last May,
forcing federal officials to scramble for other stopgap funding of $600 million, a good deal short of the $1.9 billion that the Administration sought. “We find ourselves in a rare moment where we have advance warning on a disease,” a White House official blogged last April. “However, Congress continues to do nothing about the emergency funding.”

Public health officials say that recent research now convinces them that Zika virus “causes microcephaly”—a link that was considered likely but not conclusive a mere few months earlier. “No single piece of evidence provides conclusive proof that Zika virus infection is a cause of microcephaly and other fetal brain defects,” noted Tom Frieden, director of the Centers for Disease Control and Prevention (CDC) in Atlanta, Ga., citing a report by Lyle Petersen, Sonja Rasmussen, and others at CDC. “Rather, increasing evidence from a number of recently published studies and a careful evaluation using established scientific criteria supports [those] conclusions.” Their analysis appeared 13 April 2016 in the New England Journal of Medicine (doi:10.1056/NEJMsr1604338).

Several sets of experiments in which mice were infected with Zika virus further support those conclusions. The virus “crosses the placenta and causes microcephaly by targeting cortical progenitor cells, inducing cell death by apoptosis and autophagy, and impairing neurodevelopment,” note Patricia C. B. Beltrão-Braga of the University of São Paulo in São Paulo, Brazil, and her collaborators there, at the University of California, San Diego, and elsewhere. Similarly, early in pregnancy, the Zika virus infects the placenta and fetal brain of mice, causing a syndrome that resembles what happens in Zika-infected pregnant women, according to Michael Diamond of Washington University School of Medicine in St. Louis, Mo., and his collaborators.

On the Zika diagnostics front, Food and Drug Administration (FDA) officials in April issued an emergency use authorization to Quest Diagnostics of Madison, N.J., for its PCR-based test for Zika virus. That test, developed by Quest subsidiary Focus Diagnostics, detects viral RNA in human serum specimens. Separately, an experimental paper-based test rapidly detects Zika-specific RNA sequences within the viral genome, according to James Collins of Massachusetts Institute of Technology in Cambridge, Mass., and collaborators there and at nearby Harvard University. With amplification, the test can detect viral RNA concentrations as low as 2 or 3 parts per quadrillion in serum samples from monkeys infected with Zika virus, these researchers report.

Meanwhile, in May, CDC officials broadened their interim guidance for Zika virus testing, recommending that public health laboratories extend such testing to urine specimens from patients suspected to be infected by the virus, while continuing to test for Zika virus in serum samples. Moreover, for instances where PCR test results are negative, IgM-antibody testing should be done to cover those cases where reduced viremia might account for false-negative results, CDC officials note.

Jeffrey L. Fox is the Microbe Current Topics and Features Editor.

NEW FROM ASM

Point-of-Care Workable in Developing Countries: HPV in Self-Collected Specimens

David C. Holzman

Point-of-care testing appears workable even under highly difficult circumstances—specifically, when evaluated among women in Papua New Guinea, who are at risk for becoming infected with human papillomavirus, a cause of cervical cancer, according to Andrew Vallely of the University of New South Wales in Sydney, Australia, and his collaborators. Moreover, self-sampling by these women provides specimens that screen as accurately as do cervical samples that clinicians obtained, these investigators note. They call this finding “critical” for developing same-day, screening-and-treatment procedures for women in this and other developing...

Evaluating self-collected samples was a critical milestone towards enabling same-day screening and treatment, which is needed in high-burden, low-income countries such as Papua New Guinea, Vallely says. When such patients leave clinics, typically it becomes difficult or impossible to find them again for follow-up treatments.

“Most studies showing this level of comparability at the viral detection level between self-collected and cervical samples have translated to comparable sensitivity for pre-cancer.”

Papua New Guinea has a very high burden of cervical cancer, Vallely says. The rate of new cases is six to seven times higher than in Australia and New Zealand, and mortality is around 14 times higher, making HPV-associated cancers a leading cause of premature death for this small nation.

David C. Holzman is a freelance writer in Lexington, Mass.

RESEARCH ADVANCES

Phages Form Liquid Crystals, Shaping P. aeruginosa Biofilms

Shannon Weiman

Through a novel mechanism, bacteriophage particles link with the surfaces of Pseudomonas aeruginosa cells and other nearby polymers to form tenacious biofilms, including those that form within the lungs of cystic fibrosis (CF) patients, according to Paul Bollyky of Stanford University in Stanford, Calif., who spoke during the Bay Area Microbial Pathogenesis Symposium last March in San Francisco. Bacteriophage that reproduce within P. aeruginosa are released and can assemble into liquid crystal structures that surround and protect these bacteria as part of larger biofilms, according to Bollyky, Patrick Secor, William Parks, and their collaborators. Details describing some of this research appeared November 11, 2015 in Cell Host & Microbe (doi:http://dx.doi.org/10.1016/j.chom.2015.10.013).

In viscous environments, P. aeruginosa cells upregulate their expression of filamentous phages as the cells begin to form biofilms, according to Bollyky. Within the lungs of CF patients, these long, negatively charged viruses interact with a broad variety of host and microbial polymers, including DNA molecules, mucin, and hyaluronan, forming stable liquid crystals as part of a larger and heterogeneous biofilm matrix. This structure confers multiple fitness advantages on the P. aeruginosa cells, helping to explain how biofilms enhance their pathogenic properties.

“One of the canonical features of biofilms is their ability to adhere to surfaces,” says Bollyky. “Filamentous phages make structural contributions to biofilms that increase adhesion.” In addition, the phage and bacterial cell-based liquid crystal structure increases the viscosity of mucosal secretions, a hallmark symptom for CF patients. Thus, P. aeruginosa biofilms are very difficult to dislodge from the lungs of such patients, obstructing airways.

Liquid crystals also retain water, protecting P. aeruginosa cells against drying out while promoting their survival and transmission. “The transmission of P. aeruginosa from one CF patient to another can occur through aerosols or contaminated surfaces, and desiccation tolerance is thought to be critical to transmission,” says Bollyky. Indeed, highly transmissible P. aeruginosa isolates harbor filamentous prophage capable of generating such liquid crystal biofilms.

These structures also protect bacteria against some types of antibiotics by sequestering positively charged drugs such as aminoglycosides within the negatively charged matrix, helping to explain why bacterial pathogens in biofilms resist treatment with such drugs, according to Bollyky. The matrix may also protect pathogens within them against host innate immune defenses by similarly sequestering cationic antimicrobial peptides. These data “suggest that filamentous phage contribute to the persistence of P. aeruginosa biofilm infections and may help explain how filamentous phage influence P. aeruginosa virulence in vivo,” he says.
He notes that particularly virulent clinical isolates, notorious for causing intractable lung infections, produce far more phage than do their less virulent counterparts.

Finding liquid crystals "in a medically relevant biofilm is pretty remarkable," says James Wilking of Montana State University in Bozeman, a physicist who works on biofilm structure but was not involved in this work. Phages might be playing comparable roles for other clinically challenging biofilm infections involving other gram-negative bacterial pathogens, such as Escherichia coli or Vibrio cholerae, both of which also produce filamentous phage, Bollyky points out. Adds another biofilm expert, Scott Rice of Nanyang Technological University in Singapore, "There are reports that filamentous phage increase biofilm formation for plant pathogens. [The Bollyky] study is a very nice confirmation of the physical mechanism."

Shannon Weiman is a freelance writer in Boulder, Color.

NEW FROM ASM
New Route of TB Transmission Discovered

Scientists have identified a new route of tuberculosis transmission, via the anal glands of mongoose that live in northern Botswana and northwestern Zimbabwe. The secretions are an oily substance where the hydrophobic bacterium, Mycobacterium mungi, resides. First author Kathleen Alexander of Virginia Tech in Blacksburg, Va., says the findings have implications for TB outbreak potential among wildlife and livestock: "We need to be aware of the diversity of ways that TB can be transmitted. Tuberculosis is a huge burden for the agriculture sector and environmental transmission between wildlife and livestock is an increasing concern." The research team, headed by Mitchell Palmer of the National Animal Disease Center in Ames, Iowa, plans to study the genetic characteristics that grant M. mungi this new route of transmission.


NEW FROM ASM
Arginine Disrupts Oral Biofilms and Shifts Bacterial Makeup

Supplementation of L-arginine may increase oral health, finds a collaboration of scientists from Sichuan University in Chengdu, China, the University of Pennsylvania in Philadelphia, Pa., and Colgate-Palmolive Technology Center in Piscataway, N.J. When added to a multispecies biofilm, arginine promoted growth of the arginolytic Strep-tococcus gordonii over caries-causing Streptococcus mutans, and led to an increase in biofilm pH. First author Jinping He and senior author Hyun Koo also reported that arginine treatment repressed S. mutans expression of genes associated with exopolysaccharide and bacteriocin production.


NEW FROM ASM
No Effect of Triclosan on Human Microbiome

Triclosan found in household products has no major effect on gut microbiome composition, report researchers at Stanford University in Stanford, Calif., and Cornell University in Ithaca, N.Y. Senior scientist Julie Parsonnet and first author Angela Poole led a team to test the effects of the commonly used microbicide on microbiota and endocrine function in adults. Their double-blind, randomized, crossover study found triclosan exposure did not have a significant impact on the oral or gut microbiome or on a panel of metabolic markers. This study, published in mSphere, will be followed by one investigating the microbial and growth effects of triclosan in babies and pregnant women.


NEW FROM ASM
Cystic Fibrosis Infectious Bacterium Evolves in Bursts

The opportunistic bacterium Burkholderia multivorans evolves and adapts in bursts to survive in the lungs of cystic fibrosis (CF) patients, as described by first author Inêz Silva in an mSystems report. The research team, led by Leonilde Moreira, examined a series of isolates collected from a single CF patient over 20 years. Sequence and analysis of 22 B. multivorans isolate genomes showed that new lineages evolved mainly by mutations in genes with regulatory or signaling roles, and in genes involved in metabolism. “This dynamic suggests that monitoring these evolutionary and molecular patterns could be used to design responsive therapies designed to limit population diversity and disease progression,” says Moreira.

Silva IN, Santos PM, Santos MR, Zlosnik JEA, Speert DP, Buskirk SW, Bruger EL, Waites CM, Cooper VS, Moreira LM. Long-term evolution of Burkholderia multivorans during a chronic cystic fibrosis infection reveals shifting forces of selection. mSystems. Published online 24 May 2016.
A Microbial Clue to Namib Desert Fairy Circles

New research published in Applied and Environmental Microbiology proposes that microbial phytopathogenesis plays a role in “fairy circle” (FC) formation in the Namib Desert. The southwestern African desert contains FC, which are circular deadened areas surrounded by tall grass rings. First author Andries Van der Walt, working with senior scientist Jean-Baptiste Ramond of the University of Pretoria in Pretoria, South Africa, compared microbial makeup of two types of FC soil and control soil samples using high-throughput sequencing. The two FC types had largely different microbial communities, but did share 9 bacterial, 1 archael, and 57 fungal phylotypes in common, including several known plant pathogens. The authors suggest that these plant pathogens may aid in formation or maintenance of the circles, but acknowledge more work must be done before a causative link is made.


Improving Infection Antibiotic Susceptibility Predictions

Can clinicians predict antibiotic resistance characteristics of current urinary tract infections from previous infection history? Researchers from the Rambam Health Care Campus in Haifa, Israel, have addressed this question with an analysis of nearly 20,000 paired positive urine cultures. The scientific team compared resistant cultures against a patient’s previous cultures and prevalence of the same resistance within the general population. Previously resistant cultures were predictive of the current isolate resistance phenotype, but probability varied with the type of resistance. First author Yaakov Dickstein and senior author Micah Paul concluded that culture results within the previous 6 months were informative for most resistance phenotypes outside of carbapenem-resistant enterobacteriacae (CRE), for which CRE cultures older than 6 months remained associated with current CRE status. The results should help improve efficacy for empirical antibiotic prescription.


New Tools to Research Novel Virus

Newly generated antibodies against human hepegivirus 1 (HHpgV-1), also known as human pegivirus 2 (HPgV-2), will help researchers learn more about this flavivirus, which was first detected in Fall 2015. First author Katie Coller and senior author George Dawson tested seroprevalence using the antibodies, and observed a much higher seroprevalence of HPgV-2 antibodies among those coinfected with hepatitis C virus (HCV). This confirms previous suggestions that HPgV-2 has both a similar mode of transmission as HCV and indicates there may be similar factors that put people at risk for both viruses. These new research tools, from Abbott Laboratories in Abbott Park, Ill., will complement molecular tests to provide a clearer picture of the HPgV-2 infectious cycle.


Viral Infection Studied over 15 Years

How do chronic infections change over time? This is the broad question addressed in recent research published in the Journal of Virology. In their study, a team of scientists headed by Fabio Luciani from the University of New South Wales in Sydney, Australia, investigated a hepatitis B virus (HBV) infection at different time points over a 15-year infection in a single patient. Viruses were sequenced using Pacific Bioscience single-molecule sequencing technology, which allows full-length HBV genomes in a single read. First author B. D. Betz-Stablein used this technology to show that viral deletion variants missing internal genomic content (called splice variant HBV genomes, or spHBVs) are more diverse than previously measured. The team also found that most fixation mutants occurred in the genomic region containing the HBV reverse transcriptase, which is a region known to contain resistance variations, and that evolution of resistant variants changed with the therapeutic regimen.

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Quality Annotations, a Key Frontier in the Microbial Sciences

With genomic sequencing expanding so rapidly, gene annotation lags—posing challenges to catch up while correcting errors as needed

Valérie de Crécy-Lagard

For microbiologists, the sequencing of the genome of the bacterium *Haemophilus influenzae* in 1995 was a pivotal event, one that transformed the microbiological research enterprise. More than 20 years later, with the genomes of some 85,000 organisms sequenced, including about 70,000 bacterial species, whole genome sequence (WGS) information is being used to design, conduct, and analyze vast numbers of experiments. There is no going back.

This sequencing effort cost several billion dollars, and the main rationale behind this massive investment was that information from WGS would not only accelerate basic research in biology but also bring practical benefits in medicine, agriculture, and other fields linked to the use of living organisms. Some of these benefits are being realized.

However, because biological functions are currently correctly predicted for less than half of the collective proteome, we are not yet fully benefiting from this large investment. About 20% of sequenced genes, called ORFans, are not yet linked to any function, while another 30% are assigned only very general functions. Further, at least 20% of tentatively assigned functions prove wrong, meaning considerable resources are spent on correcting annotations and following false leads. The situation does not appear to be improving. Thus, as DNA sequencing costs fall, more and more incorrect annotations are being propagated.

With so many genes being of unknown function or wrongly annotated, this step is turning out to be the Achilles heel of the genomic revolution. Solutions are available. The gene annotation community continues to develop strategies and tools to improve annotations. Because many of these tools are based on comparative genomics methods, they are more efficient as more genomes become available. The time is ripe to combine quantity with quality. However, doing so will require coordination, specific funding, and more active participation of experimentalists.

**Estimating Known and Unknown Proteins within Genomes**

Estimating proteins with known functions within a genome is a challenge. Some pipelines consider that the biological function of a protein is known once any general feature such as an ATP binding site or a transmembrane domain is identified. However, most biologists agree that although such information is useful, it is too broad and incomplete. Other experts say that both biochemical and in vivo evidence is needed for each gene before its function can be considered “known.” However, meeting this high standard appears to be feasible for only a few members of any given protein family, and hence can be met...
for a portion, “epsilon,” of the proteome. For example, of the nearly 60 million proteins currently in UniProt, only about 1.5% have functions that can also be linked to experimental evidence.

A more practical definition, the one used here, is that a function is considered known when (1) a precise molecular role is assigned (for an enzyme, a four-digit Enzyme Commission [EC] number) and (2) this role is placed in a biological context (for an enzyme, a metabolic pathway). These two criteria can be inferred from available experimental data and/or in silico information.

The likelihood of knowing protein functions for a particular organism also varies greatly with its genome size as well as knowledge about its other properties. For an intracellular bacterium with a reduced genome such as *Mycoplasma genitalium*, 60% of the genes can be linked to a function. For *Escherichia coli*, the estimate is for more than 70%. For a less extensively studied bacterium with a large genome, such as *Myxococcus xanthus*, fewer than 30% of the genes are of known function, while a general function can be assigned to another 36%. Another approach for estimating proportions of known gene functions within a genome involves considering how many proteins contain domains of unknown function and then subtracting them from the total. However, this approach grossly overestimates our current knowledge when compared to more stringent definitions of known function.

In sum, even for the best studied free-living organisms, at most 70% of proteins are considered of known function, and this number drops to roughly 30% for less-studied microorganisms with large genomes.

**Strategies for Improving Genome Annotation**

For various reasons, many genes are incorrectly annotated (Table 1). Fixing these mistakes requires different solutions with varying degrees of difficulty. For example, problems with naming should be relatively easy to fix. The Gene Ontology Consortium develops systems for consistent naming of gene functions, and there is the older Enzyme Commission number (EC number) system for assigning numbers as a way of classifying enzymes, based on the reactions that they catalyze.

Some efforts to annotate genomic sequences never make it into the databases, leaving the false impression that particular genes are unknown. Requiring authors to deposit annotations in UniProt when they publish their work is one remedy. Another is to suggest that they use tools that query PubMed by sequence similarity searches such as Seq2Ref or PubServer.

Many mistakes are due to poor annotation propagation, particularly when dealing with fusion proteins. However, annotation pipelines have improved and added domain-based criteria that can catch such mistakes. Other mistakes that derive from experimental errors are more difficult to fix. However, they could be flagged early as dubious if other types of evidence are used. The most common mistakes are found in the misannotation of paralogous family members as discussed below, and much more caution should be used to annotate those families. Finally, more systematic practices in annotating genomes would go a long way toward improving matters. Recommendations include (1) establishing highly curated reference genomes; (2) systematically reviewing annotated genomes; (3) requiring genome depositors to follow strict guidelines; (4) maintaining constant communication between sequence depositories and curation hubs; (5) routinely comparing annotations of closely related organisms; and (6) adding a family membership identifier when available to “unknown” annotations. The systematic implementation of these methods and guidelines by the most active players in the annotation field has started, but further coordination is needed to make sure chaos is not constantly recreated and to take a collective advantage of the individual strengths of each annotation platform. Many questions, such as who generates and curates the gold standard genomes or how often genomes should be reannotated in curated depositories like the NCBI RefSeq, remain to be answered.

To improve annotations, we need to ground functional calls within a biological context. For example, if the gene encoding only one enzyme of a three-step pathway is found in a genome, either the annotation is wrong or the other two genes have not been called (Fig. 1A). Thus, placing the annotation in its biological context reveals issues requiring further analyses.

One way to check for biological context is to ask experts. This approach is being used for organisms with organized research communities and dedicated databases. For example, curators...
of the Saccharomyces Genome Database (SGD) will follow up with individual experts to check annotations. A more automatable method is to use metabolic reconstruction methods to map predicted reactions and then missing components. However, these models cover mainly core metabolism, leaving many other genes unchecked. This metabolic approach can be expanded with the subsystem model to cover more genes. An expert forms a subsystem by collecting functions into a group, which may contain the reactions of a pathway or subunits of a complex. Components in a group are linked to genes and analyzed across a set of genomes (Fig. 1B). By comparing large numbers of genomes, the subsystem technology becomes very effective in identifying annotation problems and in discovering novel functions.

**Table 1. Major causes of functional annotation mistakes**

<table>
<thead>
<tr>
<th>Type of error</th>
<th>Abundance</th>
<th>Examples and/or notes</th>
<th>Possible solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paralog: annotation transferred to a non-isofunctional member of a family</td>
<td>Very common</td>
<td>Experimental validation incorrectly propagated, see the COG0720 example (PMID: 25210598) and the generality of the problem (PMID: 20011109)</td>
<td>Flag paralog families Use synteny and motifs to disambiguate paralogs Better separation of subfamilies Annotation rules (HAMAP)</td>
</tr>
<tr>
<td>Experimental mistake</td>
<td>Rare</td>
<td>The tRNA modification enzyme TsaD/YgjD was misannotated as a glycoprotease (Gcp) for years due to an experimental mistake (PMIDs: 21183954 and 21285948).</td>
<td>Detective work Gold standard reference genomes Metabolic reconstruction</td>
</tr>
<tr>
<td>Fusion (only one function called or function miscalled)</td>
<td>Very common</td>
<td>Most fusions proteins are incorrectly annotated</td>
<td>Check domain organization Size check Synteny analysis Annotation rules (HAMAP)</td>
</tr>
<tr>
<td>Classified as unknown but known</td>
<td>Very common</td>
<td>Experimental validation not captured or not propagated. For example the function of CT611 as a was published in 2007 (PMID: 17645794) but is not yet captured in UniProt (O84617).</td>
<td>Require authors to input novel function in UniProt at publication Text mining tools Seq2Ref, PubServer Manual curation Annotation rules (HAMAP)</td>
</tr>
<tr>
<td>Multiple functions not captured</td>
<td>Common</td>
<td>Also called moonlighting, these are different from fusions, as the same domain carries several reactions. See the COG0720 example (PMID: 25210598) and <a href="http://www.moonlightingproteins.org/">http://www.moonlightingproteins.org/</a></td>
<td>Same as above, but more difficult as not all members of a family might have multiple functions</td>
</tr>
<tr>
<td>Spelling mistakes or incorrect names</td>
<td>Common</td>
<td>Ureidoglycolate hydrolase, amidohydrolase, lyase genes (PMID: 24107613.)</td>
<td>Controlled vocabularies</td>
</tr>
</tbody>
</table>

**Critical Step: Avoiding Incorrect Annotation Transfers**

Deciding to transfer a function that was experimentally validated for one gene in one organism to another gene in a different organism is the critical step in annotation. The ideal case is to assign joint membership to an isofunctional protein family with members that can be separated from all other sequenced proteins by a sequence similarity threshold (Fig. 2A).

However, most protein families are not isofunctional but contain members that diverged in function. Unless the family can be clearly split into subgroups with each component having an experimental validated member in each subgroup (Fig. 2D), systematically transferring annotations within the family will lead to annota-
tion mistakes (Table 1). Because most families are not isofunctional, overannotation is a common error, and fixing these problems requires more work than would treating them as unknowns. Cases where a few mutations change function or proteins have dual functions (“moonlighting” proteins) (Fig. 2H) will always be difficult to annotate automatically. These cases require focused bioinformatics and experimental studies. The development of specific annotation rules created with expert input on a family-per-family basis such as the UniProt HAMAP rules could be one of the methods used to capture and propagate complex annotations. In short, different types of information need to be integrated to develop reliable annotations (Fig. 3, right side).

In Silico Discovery of Functions

Comparative genomic methods can also reduce “unknowns” by generating general or even specific predictions that can be experimentally validated (Fig. 3, left side). These approaches should become part of the experimentalist toolbox to supplement the BLAST sequence similarity search tool that is often the only bioinformat-
FIGURE 2

A Isofunctional and at least one validated member

B Isofunctional and no validated member

C Isofunctional and erroneously validated member with different function

D Not isofunctional with subfamilies all with validated members

E Not isofunctional with subfamilies with only a subset with validated members

F Not isofunctional with subfamilies with no validated member

G Not isofunctional with validated members

H Not isofunctional with validated members with different functions

I Not isofunctional with no validated member

Complexity of the annotation transfer process. Proteins of a given families are annotated by transferring the function from a black or gray functionally characterized member that is in the closest encompassing circle (representing a group separated from the rest of the proteome by reciprocal blast hits, orthology/paralogy or similarity networks). In this figure, the assumption is made that the correct annotations of all proteins are known, a situation that clearly does never occurs but is used here as a pedagogical tool. Families (A) and (D) are ones were the annotation transfer should not generate mistakes. The (C) example shows a case where one of the two annotations (black or grey) is wrong and further analysis is required to annotate the family. The (E) families are the ones that are often erroneously annotated by over-annotation, by transfer of the annotations to all subgroups and not just to the red one. In this case, the over-annotations can be corrected by limiting them to the correct subgroups. (G) and (H) are the really difficult cases that require a combination of tools to identify and resolve them and it is not always possible to do so. (B), (F) and (I) are all families of unknown function, but the ease and accuracy of their annotation once the function of one member is discovered will be very different.
structure data to predict gene functions (Fig. 3). The power of the approach lies in combining different types of associations.

Because the functional space to explore is so large and uncharted, predicting a precise molecular function for an unknown gene family is not trivial and can take years with trial and errors, combining in silico and wet-lab experiments. However, even if the molecular function is not always determined, using comparative genomic approaches will generally narrow the functional space.

Comparative genomic methods are extremely effective in solving missing gene problems. Indeed, no genes are yet known for about 2,000 “orphan enzymes,” that is, with “globally missing” genes. Also, the availability of WGS from diverse organisms has revealed that many organisms used enzyme families that are different in sequence and even chemistry from the canonical pathway found in the reference organisms. These are often found after a “locally missing” gene case is identified in a subsystem analysis (Fig. 1). Because the space is reduced to a set of unknown genes in a specific set of genomes, comparative genomics methods can narrow it down to a handful or even one candidate for a globally or locally missing gene. Very strong candidates can be found within a few hours using these methods once the “missing gene” problem is identified.

To take better advantage of our collective knowledge, it will help to train more experimentalists in comparative genomics. Platforms for mining multiple types of association are available, the most popular being the STRING database, which is user-friendly and captures data from multiple organisms. Other databases such as EcoliNet for E. coli or Genemania are also

![FIGURE 3](image-url)
very useful. These databases sometimes correctly predict general functions but are still too rudimentary to predict specific functions. Meanwhile, gap-filling platforms to predict missing genes have been developed, even if success stories are still rare. Even so, as integrated mining tools improve and more experimentalists use them, many more “unknown” cases surely will be solved.

Capturing Collective Knowledge

Databases for annotating genes rise and fall, but now seem to be consolidating. Some are organism-specific while others are more integrative. It is important that no single player monopolize this field, different databases communicate, funding for them be set aside and have continuity, and database curators and experimentalists interact more fully.

Biologists have split views on functional annotations—realizing that they are far from perfect but sometimes blindly trusting them for annotated genes outside their specialty. Also, some experimentalists spend time a lot of time fixing annotations for their own research without troubling to incorporate those corrections into shared databases. One remedy is to contact UniProt curators through the help links found on any of their protein pages.

In general, biologists should try to better understand the annotation process. Although training in bioinformatics is widely available, such classes are not mandatory and enrollment re-

AUTHOR PROFILE

de Crécy-Lagard: Her Lab, a Zoo; Her Focus, Unwavering

In 12th-grade biology, Valérie de Crécy-Lagard saw a metabolic map of a cell, and found it “totally mind-boggling,” she says. Years later, “the first thing I did when I had my own office was to get the metabolic map up on my wall.” Such a map hangs in her office at the University of Florida, where she is a professor of microbiology and cell science.

The focus of her research is on the accuracy of translation, metal homeostasis, vitamin metabolisms, and metabolite repair, according to de Crécy-Lagard. She relies on microbial genetics to test hypotheses she derives from data mining. Favorite microorganisms include bacteria such as Escherichia coli and Acinetobacter baylyi, the archaeon Haloferax volcanii, and fungi such as Saccharomyces cerevisiae, she says. “My lab is a zoo.”

de Crécy-Lagard, 51, was born in France, but traveled throughout her childhood because her economist father was a diplomat who later worked for a multinational company. Thus, with her family, she spent time in England, Romania, and Italy as well as France before college. Her mother was a student of Spanish, while her sister is a writer.

Earlier, de Crécy-Lagard studied math, and considered a career in medicine. “I wanted to be an M.D. until I was 13 or 14, and then realized I would be much happier in an ivory tower,” she says. She earned her B.Sc. in general science in 1987 at the Ecole Polytechnique at Palaiseau, her Ph.D. in biochemistry and microbiology in 1991 at the University Paris VII, and from 1991 to 1993 did postdoctoral research at the National Cancer Institute in Bethesda, Md.

“I started a Ph.D. because I realized I was very intellectually gullible,” she says. “I thought a few years of research would teach me to be a more critical thinker. I had not predicted at all I would catch the research bug. I think the fact that I was a math/physics major and learned microbiology/biochemistry late helps me. I follow the logic of genomic clues, and this has allowed me to figure out things that often look simple after the fact.”

With her husband, a biotech entrepreneur, de Crécy-Lagard returned to the United States in 1998, and now holds dual U.S.-French citizenship. “We came to the U.S. with the children (a son and daughter, now grown) and a few suitcases,” she says. “We took a map and chose a place where the weather was nice, and the science was great.” They lived in San Diego for five years before moving to Florida in 2004.

de Crécy-Lagard plays tennis daily. “When I am on the tennis court, I forget about the lab, grants, and experiments, and just focus on this little yellow ball,” she says. She also likes art deco architecture and mystery novels in both English and French. “I get totally lost in books, and the outside world would be suspended,” she says. This same focus envelops her when scanning genomes, she adds. “The world could crumble around me without notice, and I could spend the whole weekend on the computer.”

Marlene Cimons
Marlene Cimons lives and writes in Bethesda, Md.
mains spotty. Meanwhile, incentives are needed to make it easier to correct annotations in databases. This effort could include publishing corrections in journals in a format that could be captured easily by databases. Also, granting agencies should fund these efforts explicitly. Finally, databases should recognize and hold accountable those scientists who suggest such changes and corrections.

**Conclusion**

The great number of available WGS will only increase, making comparative genomic methods very efficient to improve annotations of both known and unknown genes. The systematic use of these methods would increase the number of reliable annotations by at least 30% if not more. This would improve the interpretation of systemwide experiments in any organism and would also focus the experimental effort on the genes that cannot be predicted with high confidence by in silico methods or that are truly of totally unknown function. Because of the size and importance of the task, there needs to be an increase in the number of players involved in functional annotations with more professional curators and a greater involvement of the biologist community.

**Acknowledgments**

I thank Gilles Basset (U. of Florida), Najib El-Sayed (U. of Maryland), Jamie Foster (U. of Florida), Susan Gottesman (National Cancer Institute), Sveta Gerdes (Fellowship for Interpretation of Genomes), and Claire O’Donovan (UniProt) for their invaluable edits, comments, and suggestions. I also thank Alvaro Glavic for inviting me for a two-week visit to Chile that liberated the time to focus on writing this manifesto.

Valérie de Crécy-Lagard is a professor in the Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences and Genetics Institute, University of Florida, Gainesville.

**Suggested Reading**


Percudani R, Carnevali D, Puggioni V. 2013. Ureidoglycolate hydrolase, amidohydrolase, lyase: how errors in biological databases are incorporated in scientific papers and vice versa. Database 2013.


Whole-Genome Sequencing Is Taking over Foodborne Disease Surveillance

Public health microbiology is undergoing its biggest change in a generation, replacing traditional methods with whole-genome sequencing

Heather A. Carleton and Peter Gerner-Smidt

About 1 in 6 people are sickened with foodborne diseases each year, and for the most part those illness bouts are a nuisance and self-limiting. However, for some vulnerable populations, severe foodborne illnesses can require hospital care and may even lead to death. Once an individual becomes sick enough to visit a physician, he or she typically collects a stool sample to send to a clinical microbiology laboratory for testing and diagnosis. If the clinical laboratory identifies an enteric pathogen, the physician is notified.

Clinical laboratories will usually also send bacterial cultures or samples to local public health laboratories. What goes on there has no direct consequence for patients and their doctors unless a particular patient is suspected to be part of an outbreak. In the public health laboratory, the isolate will be characterized and subtyped in the PulseNet system, the US national network that is the primary early warning laboratory system for foodborne outbreaks. Patients who are involved in outbreaks typically are contacted by an epidemiologist asking about what foods might be responsible for the outbreak.

The PulseNet testing method to screen samples changed very little until recently, when whole-genome sequencing (WGS) began to replace pulsed-field gel electrophoresis (PFGE), which was used almost exclusively for the past 20 years. This network is coordinated by a team of microbiologists at the Centers for Disease Control and Prevention (CDC) in Atlanta, Ga., who work closely with microbiologists in more than 80 local, state, and federal public health and food regulatory laboratories. One of their main tasks is to collect molecular data characterizing foodborne bacteria that infect patients along with demographic data omitting personal identifiers, and then to submit that information to a national database.

Similar data characterizing microorganisms isolated from foods and food production facilities are also submitted to PulseNet. To detect outbreaks, microbiologists at the CDC as well as state and local health departments look for trends in these data. Are a higher number of similar molecular fingerprints being generated in a particular region of the US than at the same time during past years? Has the fingerprint never been seen in the PulseNet database except for the last month or so? By analyzing these types of clues, the PulseNet teams can determine whether the bacteria being isolated are linked to outbreaks, and then they can communicate this information to epidemiologists who lead investigations of suspected outbreaks.

Public Health Microbiology and Molecular Surveillance Workflows

Microbiologists working at public health laboratories typically will identify the genus and species

SUMMARY

➤ Whole-genome sequencing (WGS) is beginning to replace pulsed-gel electrophoresis (PFGE) for subtyping of foodborne pathogens from stools and other specimens for outbreak surveillance.

➤ For more than 20 years, PFGE, a molecular fingerprinting technique that can be adapted to determine the subtype of almost any bacteria, was the principal method for detecting and investigating foodborne disease outbreaks in the United States.

➤ Investigators at PulseNet are working with other investigators in and outside the United States (US) from public health, food regulatory laboratories, and universities to build standard WGS multilocus sequence typing (MLST) databases for analyzing common foodborne pathogens.

➤ The development of metagenomic sequencing-based tools combining diagnostics and surveillance, a challenging task, could soon enable public health investigators to detect outbreaks much earlier and likely shortly after the first patients begin visiting their physicians.
of the bacteria being tested. However, they may also further characterize the samples they are analyzing, depending on the pathogen in question. For example, they may serotype *Salmonella*, Shiga toxin-producing *Escherichia coli* (STEC), *Shigella*, *Vibrio*, and *Listeria*; characterize virulence determinants carried by diarrheagenic *E. coli* pathotypes (STEC, enteropathogenic *E. coli* [EPEC], enterotoxigenic *E. coli* [ETEC], enter-aggregative *E. coli* [EAEC], enteroinvasive *E. coli* [EIEC]), and *Shigella*; or *Vibrio* spp., and may determine the antimicrobial susceptibilities of isolates.

The assays used to characterize such samples vary in complexity and can include a large spectrum of phenotypic and molecular tests. They include observing growth of the bacteria on different types of media, fermentation and biochemical reaction tests, agglutination with diagnostic antisera, immunofluorescence, cell culture assays, protein electrophoretic assays, and PCR. Each isolate typically is characterized using multiple independent assays. If the isolated pathogen is a *Salmonella*, STEC, *Shigella*, *Listeria*, *Vibrio*, or *Campylobacter*, it likely will also be subtyped with a highly discriminatory DNA fingerprinting method to determine if it could be part of an outbreak.

For more than 20 years, PFGE, a molecular fingerprinting technique, was the principal method for detecting and investigating foodborne disease outbreaks in the United States. PFGE is the only restriction fragment length polymorphism (RFLP) procedure from the 1970s and 1980s still in wide use. It entails the use of restriction enzymes to cut bacterial genomes into 10–30 large pieces (10–500 kb) that are separated in agarose gels that are exposed to alternating, or pulsed, electric fields under conditions that separate the fragments according to their size. The banding pattern provides a characteristic pattern, or “fingerprint,” for each bacterial strain that the microbiologist compares to those for other isolates to detect whether an outbreak is going on.

This method survived so long because it can be adapted to subtype almost any bacteria and standardized to compare results from different laboratories, and proved highly efficient in detecting and investigating outbreaks. Until WGS was introduced, no other subtyping method had all these characteristics. Moreover, when PFGE does not sufficiently discriminate between isolates, the method can be supplemented with other pathogen-specific assays. Most recently, multilocus variable number of tandem repeats analysis was used for outbreaks involving pathogens such as *E. coli* O157 and *Salmonella enterica* serovars Typhi murium and Enteriditis.

With PFGE still in wide use, public health laboratories rely on several complex, dated, and therefore expensive pathogen-specific methods that take specialized expertise to perform and interpret. Additionally, the turnaround times for characterizing foodborne pathogens in many public health laboratories range from four days to several weeks or months, depending on the workflow for a particular pathogen. Therefore, a method that could simplify and accelerate such testing is highly desirable, especially if it is cost-efficient and could replace these older technologies. WGS has the potential to do exactly that (Fig. 1).

### Public Health Microbiology Embracing Whole-Genome Sequencing

Next-generation sequencing (NGS) technology is drastically reducing the cost and time needed to sequence bacterial genomes, making this analytic approach feasible for both reference and subtyping purposes at public health laboratories. Instead of relying on multiple workflows to identify pathogens and their serotypes, virulence factors, antimicrobial resistance factors, and molecular fingerprint on pulse gels, much of this information can be extracted from WGS data. Additionally, NGS can reduce turnaround times to a mere 2–4 days.

The genetics underlying many phenotypic tests of bacterial pathogens are known, and PCR assays already replaced many of these tests. WGS can easily, in turn, replace PCR or those older tests. Indeed, many WGS-based analyses are already freely available to the scientific community and could be made even more useful if applied to public health.

For example, the Center for Genomic Epidemiology at the Danish Technical University (https://cge.cbs.dtu.dk/services/) is a particularly good source for several such tools, including tools to detect antimicrobial resistance (ResFinder) and virulence genes in *E. coli* (VirulenceFinder), to determine serotype of *E. coli* (SerotypeFinder), and to characterize plasmids in *Enterobactericeae* (PlasmidFinder). The University of Georgia
hosts a valuable tool for serotyping of *Salmonella* (http://www.denglab.info/SeqSero).

The serotyping tools for *E. coli* and *Salmonella* determine the serotype from genes that encode the O and H antigens from assemblies or raw sequence data, and the serotypes are therefore with few exceptions fully compatible with the existing serotyping schemes and have the advantage of being able to type rough isolates that are untypeable by traditional agglutination tests. However, the drawback of using these Web services is that the user can use only one tool at a time, even though sequences of multiple isolates may be batched. The number of different traditional tests will be replaced by the same number of queries of the tools.

For subtyping, WGS-based approaches provide better resolution to identify relatedness of isolates during an outbreak than almost any other method, including PFGE (Fig. 2). Rather than comparing isolates by their pattern of 15–30 differently sized bands, isolates can be compared across millions of base pairs by doing comparisons of single-nucleotide polymorphisms (SNPs) or gene-by-gene comparisons, such as whole-genome multilocus sequence typing (wgMLST).

Both analytical approaches are being used for investigating outbreaks and seem to be equally useful. Numerous SNP analyses pipelines are available in the public domain but require bioinformatics expertise to perform and generally require a priori knowledge about the isolates being

*FIGURE 1* PulseNet, the national molecular subtyping network for foodborne disease surveillance, is replacing older subtyping methods like pulsed field gel electrophoresis (PFGE) with whole-genome sequencing (WGS).
sequenced. This requirement does not hold for wgMLST because it relies on a database of all genes, or loci, generated from multiple, diverse reference genomes. These wgMLST databases are built to provide maximum discrimination for all isolates of a given genus or species. Isolates from different outbreaks of the same species can easily be compared using wgMLST at variance with SNP analysis, which is reliable only in a narrow phylogenetic context. Isolates from different outbreaks that were investigated using different SNP reference strains cannot readily be compared. For these reasons, the gene-by-gene approach is the clear winner for national and international surveillance of foodborne pathogens.

In the United States alone, more than 60,000 enteric bacterial isolates are analyzed at local, state, and federal public health laboratories each year. Because quick turnaround times are needed to detect and investigate outbreaks, any tool developed to analyze WGS data must have a simple workflow to meet both reference and outbreak surveillance analysis needs, meaning it must be easy to operate for a public health microbiologist with little to no bioinformatics expertise.

The subtyping utility of WGS, wgMLST and PFGE in the 2014 caramel apples *Listeria* outbreak. Improved resolution of WGS over PFGE for outbreak investigations. The figure shows WGS-based similarity tree of 18 outbreak-related isolates and three isolates unrelated to the outbreak that are indistinguishable by PFGE from the outbreak isolates. Three PFGE patterns are illustrated by the colored bars to the right of the tree. By sequencing, two PFGE patterns (in red and yellow) appear to be related to each other by WGS and outbreak related; the wgMLST differences are listed per branch as median [range]. The branch that contains ≤6 alleles difference between isolates (cluster 1) contains 5 patient isolates (denoted by number) and 3 food/environmental isolates (denoted by dash(-)). This cluster was distinct from the unrelated isolates at the top of the tree by 114 allele differences, though the isolates shared a common PFGE pattern. Cluster 2 (in green) contained 10 isolates associated with the same source as Cluster 1 but a different PFGE pattern. One isolate that was collected during the same time period as cluster 2 and was the same PFGE pattern, but was clearly distinct by WGS and shown to be unrelated to the outbreak. The food and environmental isolates were sequenced by FDA and the FDA sponsored GenomeTrakr network.
tools separately is inefficient, it is far better to include all tools in a single analytical workflow for both reference characterizations and subtyping. Such a system must freely import and export data to other systems, including surveillance databases and laboratory information management systems. To meet the needs of public health laboratories, typically such versatile systems either are built in-house from scratch or by combining different databases with analytical software programs because versatile commercial software packages are scarce or not fully adapted for use with WGS. However, one software package, BioNumerics, which is marketed by Applied Maths of Austin, Tex., includes both database and advanced analytical functionality including WGS analyses capabilities in its latest edition (v7.5).

Investigators at PulseNet are working with other investigators in and outside the United States (US) from public health, food regulatory laboratories, and universities to build standard wgMLST databases for analyzing common foodborne pathogens (Fig. 3). Tools that identify species on the basis of average nucleotide identity (ANI), serotyping, virulence, and antimicrobial resistance determinants, etc. In the United States a national database is housed at CDC and local databases in each PulseNet participating laboratory.
packages for identifying Campylobacter, STEC, and Clostridium botulinum are expected to follow later in 2016, and those for Salmonella in 2017, other diarrheagenic E. coli and Shigella and Vibrio in 2018, and for Yersinia enterocolitica and Cronobacter in 2019.

Global partners are also developing quality standards for raw DNA sequences and proficiency testing standards to ensure that anyone using these analytic systems can produce high-quality results that may be compared reliably on national, regional, and global scales. In 2013 PulseNet and other US public health labs began using WGS routinely for surveillance of listeriosis; this approach has led to the detection of more outbreaks, and more outbreaks have been solved. Even though the incidence of foodborne illnesses is not increasing, the technology will likely lead officials to recognize many more outbreaks caused by foodborne pathogens. This anticipated increase in reported outbreaks could prove a challenge for those states whose public health departments are not prepared to take on larger workloads.

The Future of Public Health Microbiology
Clinical laboratories increasingly are relying on multiplexed molecular panels to test stool specimens for enteric pathogens, determining within a matter of hours bacterial, viral, and parasitic pathogens with high sensitivity and specificity. To clinicians, these tests are a huge step forward because they can detect pathogens that previously rarely were looked for, including diarrheagenic E. coli in addition to STEC, viruses, and parasites. Thus, they provide actionable results and help with the management of patients.

However, because such tests do not require the culturing of microorganisms, those labs are no longer setting isolates aside for surveillance. To address this issue, measures are being put in place to maintain the flow of isolates from positive tests at clinical or public health laboratories now using those culture-independent testing methods. However, this approach will not be sustainable in the long-term. Hence, new metagenomic sequencing-based pathogen detection and subtyping tools are being developed to characterize stool specimens.

The development of these metagenome sequencing-based surveillance tools is not a trivial task. Any approach needs to be low cost to stay affordable for public health labs to use and must also provide epidemiologically meaningful subtyping capabilities during disease outbreaks. Meeting this latter need can prove challenging when identifying pathogens in the context of the normal enteric flora, which contains many microorganisms that closely resemble enteric pathogens. Data from WGS of pure cultures will be critical to develop these metagenomics surveillance tests, as well other advances in sequencing and bioinformatics technology.

As these technologies advance, the day will come when individuals with foodborne diseases will visit their physicians, who will perform WGS on stool samples using instruments that directly plug into laptops or smartphones. Once sequencing is completed, the patients and their physicians will know not only which pathogen caused a particular illness but will also have detailed information about what virulence and antimicrobial resistance factors it carries. Finally, those analytic results will also automatically be submitted to local public health authorities to determine whether individual patients are part of larger outbreaks. This way, public health investigators will be able to detect outbreaks soon after the first patients become sick—much faster than the 1–2 weeks required with current culture-based detection technologies.

Heather A. Carleton is Leader of the Bioinformatics Team and Peter Gerner-Smidt is Branch Chief in the Enteric Diseases Laboratory Branch, Centers for Disease Control & Prevention, Atlanta, Ga.

Disclaimers
The findings and conclusions in this feature are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention. Use of trade names is for identification only and does not imply endorsement by the Centers for Disease Control and Prevention or by the U.S. Department of Health and Human Services.

Suggested Reading
CDC AMD website. http://www.cdc.gov/amd/


ASM Meetings and Conferences

ASM Microbe ONLINE—Content Now Available! ASM Microbe 2016 was jam-packed with informative sessions. Gain access to audio-synced slides from the event throughout the year. You can either purchase the Track Package at $209 per track, or the Full Package at $449. Session recordings do not include the workshop program, and participation of all speakers is subject to their approval. Get your package today at www.asmeventsonline.com.

Save the Date: ASM Microbe Heads to New Orleans. Mark your calendar for ASM Microbe 2017 (June 1–5, New Orleans, La.): the premier event in the field that features cutting-edge science, world-class speakers, abundant networking opportunities, and much more. Don’t miss the rare opportunity to explore the full scope of microbiology by choosing from more than 200 engaging sessions and workshops at this unique event. For more information, visit www.asm.org/meetings.

Mark Your Calendars: 2017 Clinical Virology Symposium. Join us May 7–10, 2017, for the 33rd annual Clinical Virology Symposium in Savannah, Ga. This international symposium delves into the relationship between rapid viral diagnosis, clinical course of viral infections, and preventive and therapeutic modalities for viral infections. Don’t miss the opportunity to connect, share, and learn with your peers. For more information, visit www.asm.org/meetings.

Upcoming ASM Conferences

ASM Conferences address the needs of the diverse scientific interests of microbiologists by providing a forum for international groups of scientists to discuss their specific area of concentration. Mark your calendar for these upcoming ASM Conferences. For more information, visit www.asm.org/conferences.

ASM Conference on Streptococcal Genetics (July 31–August 3, 2016, Washington, D.C.)
5th ASM Conference on Salmonella (August 29–September 1, 2016, Potsdam, Germany)
6th ASM Conference on Beneficial Microbes (September 9–12, 2016, Seattle, Wash.)
ASM Conference on Antibacterial Development (December 11–14, 2016, Washington, D.C.)
ASM Conference on Innovative Microbial Ecology for Mitigation of Antibiotic Resistance and Bacterial Diseases (March 2017)
ASM Conference on Mechanisms of Interbacterial Cooperation and Competition (March 2017)
ASM Conference on Tuberculosis: Past, Present and Future (April 2017)
ASM Conference on Interplay of Viral and Bacterial Pathogens (ASM-ASV collaboration) (May 2017)
2nd ASM Conference on Rapid Applied Microbial Next-Generation Sequencing and Bioinformatic Pipelines (September 2017)
6th ASM Conference on Cell-Cell Communication in Bacteria (October 2017)
4th ASM Conference on Viral Manipulation of Nuclear Processes (December 2017)
ASM Public Affairs

ASM on Capitol Hill

On April 26, Kathleen Alexander, D.V.M., Ph.D., member of the ASM Public and Scientific Affairs Board (PSAB) Committee on Agricultural and Food Microbiology, represented ASM at the 22nd Annual Coalition for National Science Funding (CNSF) Capitol Hill Exhibition & Reception. Alexander presented a poster—titled “Linkages between Multidrug Resistance, Environmental Change and Human Health in Africa: Looking beyond the Usual Suspects”—and discussed her research recently published in mBio on Mycobacterium mungi, an emerging TB pathogen found in banded mongoose. Alexander also met with staff in the offices of Senator Mark Warner (D-VA); Representative H. Morgan Griffith (R-VA); Representative Louise Slaughter (D-NY); and Representative Harold Rogers (R-KY), Chair of the House Appropriations committee, to discuss her research and the importance of federal funding for the National Science Foundation (NSF).

The CNSF is comprised of more than 140 professional organizations, scientific societies, universities, and businesses whose common mission is to “support the goal of increasing the national investment in the National Science Foundation’s research and education programs in response to the unprecedented scientific, technological and economic opportunities facing the United States.” The Annual Capitol Hill Exhibition & Reception showcases the importance and range of programs and research supported by the NSF to members of Congress and their staff.

PSAB Members on the Hill to Support Zika Funding

On May 12, members of the ASM Public and Scientific Affairs Board (PSAB) traveled to Capitol Hill, met with Congressional leaders and staff, discussed FY 2017 appropriations, and advocated for passage of the administration’s $1.8 billion supplemental funding request to address the Zika outbreak. Ron Atlas, Chair of the Public and Scientific Affairs Board; James Roth, Chair of the PSAB Committee on Agricultural and Food Microbiology; Chuck Rice, Chair of the PSAB Committee on Environmental Microbiology; and Jay Grimes, former chair of the PSAB Committee on Environmental Microbiology, met with Senator Thad Cochran (R-MS), Chair of the Senate Committee on Appropriations, and staff in the offices of Senator Jerry Moran (R-KS), Chair of the Senate Agriculture Appropriations subcommittee; and Representative Harold Rogers (R-KY-5), Chair of the House Appropriations Committee.

The meetings focused on the importance of adequate federal funding to combat the current Zika outbreak and prepare for other emerging and re-emerging infectious diseases. Also discussed was antimicrobial resistance, funding for agencies of importance to microbiology, specifically the Department of Agriculture’s Agriculture and Food Research Initiative (AFRI) and microbiology broadly.

ASM Supports $1.9 Billion Emergency Funding to Combat Zika

ASM sent a letter to Congress supporting the full $1.9 billion emergency funding request to combat Zika. The $622 million proposed in the House Appropriations Committee’s Zika Appropriations Response Act is too far below the Obama administration’s earlier request of $1.9 billion for emergency Zika funding. ASM commented that underfunding Zika research and control efforts would have real public health consequences that could be avoided and that federal and state agencies must be able to respond with the best available scientific and public health resources. To read the letter, go to http://www.asm.org/index.php/public-policy/137-policy/documents/statements-and-testimony/94230-zika-5-18-16. A summary of ASM’s efforts to combat Zika can be found at http://www.asm.org/index.php/public-policy/93-policy/94194-asm-acts-to-counter-zika-virus-outbreak.

ASM Comments on Laboratory Developed Tests

On April 27, ASM joined the Infectious Diseases Society of America and the Pan American Society for Clinical Virology in authoring a letter to U.S. Food and Drug Administration (FDA) Commissioner Califf detailing joint
concerns about the FDA regulation of infectious disease laboratory developed tests (LDTs). Specifically, infectious disease LDTs have a long history of safe and effective use, and the three societies believe the risks posed by these LDTs are far outweighed by their advances and benefits to patient care. To read the letter in its entirety, please go to http://www.asm.org/images/PSAB/ASM-IDSA-PASCV-LDT.pdf.

ASM Participates in BMBL Virtual Town Hall

The National Academy of Sciences, Engineering, and Medicine solicited stakeholder input for a revision of “Biosafety in Microbiological and Biomedical Laboratories (BMBL)” beginning April 4 and leading up to a virtual town hall on May 12. Since its initial publication in 1984, the BMBL has become the cornerstone of the practice of biosafety in the United States and in many countries around the world, but this resource has not been revised since 2009. ASM was represented at the virtual town hall by Michael Pentella, member of the ASM Public and Scientific Affairs Board Committee on Laboratory Practices, and a bulk email was sent to ASM members to solicit input on this valuable resource. To view the public comments submitted, go to http://nas-sites.org/bmbl/ and click the “Virtual Town Hall” tab.

ASM Attends CHF Meeting with Senate Staffers

On May 13, ASM joined other member organizations of the Coalition for Health Funding (CHF) in a meeting with a bipartisan group of Congressional staffers, including Senate Appropriations Staff Directors Charles Kieffer and Bruce Evans. This meeting focused on the appropriations process for the remainder of the year, long term planning for public health emergencies, and included an extended question and answer period. To read more about the CHF’s history and mission, go to http://www.publichealthfunding.org/.

Membership

40-, 50-, and 60-Year Members

The following ASM members are marking 40, 50, and 60 years as members of the Society.

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Obituary

Arthur L. Koch

Arthur L. Koch, 90, passed away on May 10, 2016, from Alzheimer’s Disease. Arthur has been described as “one of the true Renaissance scientists of the past several decades.” He published on diverse topics, including metabolic rates and cell growth, antibiotic resistance, cellular evolution, gliding motility, bacterial growth and division, bacterial shape, solute transport, bacterial light scattering, adaptive responses of bacteria, the interferon dose response curve, and even problems faced by migrating sea turtles! His last paper, from 2008, is on “Stone-Age Diseases and Modern AIDS.”

Arthur served in the U.S. Navy during World War II in the Pacific. After returning he completed his chemistry B.S. (Cal Tech, 1948), and a biochemistry Ph.D. (University of Chicago, 1951). Following positions at Argonne National Laboratory (1951–56) and the University of Florida College of Medicine (1956–67), in 1967 Arthur joined Indiana University (IU) as a Professor in what was then the Department of Bacteriology, and remained at IU until retirement (1967–98). Arthur published over 250 papers, presented hundreds of seminars, and won numerous
awards, including a Guggenheim fellowship, a Rockefeller Scholar award, a Wellcome research travel grant, and a U.S. Public Health Service award.

Arthur practiced the art of mathematical biology decades before its recent rebirth. His surface stress theory, among his most prominent achievements, describes how surface tension-like forces determine bacterial shape, and how bacteria can grow but maintain cellular integrity with huge internal turgor pressure. Arthur’s seminal theoretical work in the 1980s on cell-wall growth based on equations developed by D’Arcy Wentworth Thompson (1917) is still cited. The surface stress theory applies not only to cell shape and growth, but also to autolysin regulation, cell wall maintenance, chromosome segregation, and motility.

Arthur’s book, *Bacterial Growth and Form*, summarizes his fascination with microbiology. In the preface he writes, “My most important goal in writing the book is to make accessible the relevant thinking from fields of science other than microbiology that are important to microbiology,” summing up Arthur’s career quite admirably. Foremost he was a teacher, expanding microbiological perspectives and providing theoretical bases for understanding wide-ranging microbiological phenomena. More simply, he helped and encouraged many in their research. There was practically no area of microbiology and life science in which Arthur lacked interest and understanding. Arthur trained many students through their Ph.D. and master’s degrees at Florida and later at IU. In addition, his colleagues benefitted from and enjoyed Arthur’s frequent and enlightening visits with a pile of papers and advice about their own favorite research topic. Even after retiring in 1998, Arthur was a spirited regular attendee at many IU seminar series and journal clubs and could be counted on to ask a memorable question of almost any presenter on almost any subject.

Arthur Koch was a unique, one-of-a-kind gentleman, quirky, intelligent and interested in a wide breadth of topics including science but also music, the outdoors, and his family, friends and colleagues. His absence from our department and now from the community is a loss for us all. He will be sorely missed.

Yves Brun
Clay Fuqua

*Indiana University, Bloomington*
Microbe Mentor

Lead Scientist at the USDA: Jeffrey McGarvey

What are my options outside of academia for a career?

In our second article addressing the question, “What are my options outside of academia for a career?,” Microbe Mentor reached out to Jeffery McGarvey, Lead Scientist at the United States Department of Agriculture to talk about his career in government. Check out more career profiles, like this one, on ASM’s career website (www.asm.org/careers).

Name: Jeffery A. McGarvey
Current Title, Organization: Lead Scientist, Microbiologist, United States Department of Agriculture, Agricultural Research Service, Foodborne Toxin Detection and Prevention Research Unit

Sector: Government

Degrees and Schools Attended:
B.S. University of Louisiana, Lafayette
Ph.D. University of Georgia, Athens

Describe your job and what you do on a day-to-day basis?

At the USDA, Agricultural Research Service, we partner with the agriculture industry to solve problems. My lab is funded by the USDA National Program for Food Safety, and we try to identify ways to prevent food from becoming contaminated with pathogenic bacteria, such as Salmonella enterica. We are currently trying to identify beneficial bacteria that can colonize the surfaces of crop plants in the field and prevent Salmonella from colonizing them (i.e., biocontrol agents). On a typical day I get to the lab around 7:00 AM (before my technician and postdoc) and respond to e-mails, perform my Lead Scientist duties (mostly paperwork), and get organized for the day. I am also an Editor for the Journal of Applied Microbiology and Letters in Applied Microbiology, so I also spend time reading manuscripts and finding reviewers to evaluate them.

My technician comes in at 8:30, and we go over the results from the previous day and develop a plan for the day. After that I meet with my postdoc and discuss his project results. The next few hours are spent ordering supplies and making sure everyone has what they need to do the work. In the afternoons I spend time writing papers, grants, and/or reports. I also spend a lot of time reading the literature and developing ideas for future projects. At some point I try to squeeze in some lab time for myself. Although I spend most of my time in the office, I still have some bench space and enjoy working on new projects.

How did you get into your current position? In other words, what experiences/skills did you have that made you a good fit for your current job?

I received my Ph.D. from the University of Georgia, studying the plant-microbe interactions between the plant pathogen Ralstonia solanacearum and tomato plants. I then went to San Francisco for a postdoctoral fellowship studying the human pathogenic mycobacteria (e.g., M. tuberculosis, M. avium, etc.). After the third year of my fellowship, I noticed an announcement in ASM News (now Microbe) for a job at the USDA in nearby Albany, Calif., studying Mycobacterium paratuberculosis infections in cattle, and applied for it and they hired me. After about three years they eliminated the M. paratuberculosis project and shifted our group’s focus to human pathogen contamination of produce. With my previous experience with plant pathogens it was an easy transition, and I have been working with Salmonella and other human pathogens on produce ever since.

What can students and postdocs do right now to best prepare themselves for entering your profession?

There are a lot of things students and postdocs can do to prepare themselves for a job in a government lab. First, get as much experience in different aspects of microbiology as possible. In my lab we have projects that involve cloning and sequencing genes, purifying proteins, extracting environmental DNA from diverse samples, mak-
ing monoclonal antibodies, developing in vitro diagnostic assays, isolating and identifying bacteria from environmental samples, etc. Don’t be afraid to try something new, you just may like it... Second, if you want to run your own lab, you need to publish, publish, publish. It is very hard to get a job leading a lab if you don’t have multiple, high-quality publications. Lastly, and this may sound obvious, but be nice to people. I know people who have been excluded from collaborations or have been passed over for jobs and promotions because they are difficult to work with. The golden rule applies to microbiologists too.

What is the outlook for job prospects in your field?

I think the outlook for microbiology jobs in the federal government is very positive. We currently have two vacant positions for Primary Investigators in my department and two vacant postdoctoral positions as well. At the B.S./M.S. level, we hire new people every year. One can go to www.usajobs.gov to find a full list of vacancies in the federal government. A quick search using the keyword “microbiology” just gave me over 100 vacant positions across the United States.

What is your one piece of career advice for the next generation of microbiologists?

Make yourself indispensable by knowing how to do things that others don’t. For example, a really bright and motivated technician in my department volunteered to go to training to learn how to operate our new fluorescence-activated cell sorter. He has become so proficient at it that everyone wants to have him collaborate on their projects (especially me!). Be proactive and become the expert at something, you will not be disappointed.

It is important to have a work/life balance. What is one thing you enjoy doing when you are away from the job?

Yes, it is very important to balance your work and life. During undergraduate and graduate school many of us work unreasonable hours and stress ourselves too much. For many, this hectic schedule continues during their postdoctoral fellowship and then again as new PI. Over time this will take a toll on your mental and physical health if you are not careful. You only live once, so take some time to smell the roses! I love traveling to new places and experiencing different cultures. Before I depart this world I want to visit every continent, as many times as possible. So far I have been to five continents— only South America and Antarctica remain, but not for long.

ASM’s New Career Website: Cultivate Your Career

Visit asm.org/careers for

- Professional development, volunteer, and funding opportunities
- ASM’s job board – Career Connections
- Profiles of microbiology career paths
- Articles on writing resumes, elevator pitches, networking, and more!
Reviews and Resources

BOOKS

**Metabolism and Bacterial Pathogenesis**

“Although several factors could theoretically contribute to a microorganism’s ability to colonize the intestinal ecosystem, effective completion for nutrients is paramount to success.” So the editors reference researcher Rolf Freter in their introduction to this new, integrative text. This volume highlights this truth with a biochemical focus on bacterial pathogens and the human host. This includes chapters on enteric, respiratory, urinary tract, and intracellular pathogens. Some chapters also focus attention on the role of commensal communities, such as in dental plaque or in the gut through interaction with host immunity. More species-specific topics include central carbon metabolism by *Borrelia burgdorferi*, regulation of *Escherichia coli* fimbiae by host sialic acid, and *Pseudomonas aeruginosa* metabolism during infection of cystic fibrosis patients. Though it is sparse in its figures, this is a timely and information-rich collection that should be a welcome resource for many microbiologists.

Daniel P. Haeusser
Cannisus College
Buffalo, N.Y.
Originally published in Small Things Considered,
April 14, 2016.

**Climate Change and Microbial Ecology: Current Research and Future Trends**

As one might expect, this new text nicely reviews important topics in environmental microbiology research related to the increasing trends of global climate change. Editor Jürgen Marxsen does a great job with the breadth of coverage, including chapters on viruses, bacteria, fungi, and protists alike, in a range of environments from marine, freshwater, to soil. Chapters address both the data that is known regarding alterations in biochemistry, biogeology, and community interactions due to climate change, but also highlight areas where data is particularly lacking, such as in sediments and inland waters. The first chapters also incorporate relevance to rising pathogenic risks due to climate change through alterations in abundance of cyanobacteria or *Vibrio* species, or expansion of freshwater parasite ranges. With multiple black-and-white and color figures typical of journal review articles in each chapter, this is an impressive short overview of a topic with likely broad student appeal.

Daniel P. Haeusser
Originally published in Small Things Considered,
April 14, 2016.

**Manual of Clinical Microbiology, 11th Ed.**

Many readers are doubtless already aware of this extensive and essential reference for current information and practice related to clinical microbiology. Covering organismal biology, disease characteristics, research and diagnostic techniques, antimicrobial agents, and safety practices, the recent 11th edition incorporates the latest findings, particularly the growing genomic and proteomic data available for pathogens. For general interest readers, the opening section of the first volume has excellent chapters of basic information on topics such as microscopy, molecular epidemiology, biothreat agents, and the human microbiome. The remainder of the first volume deals with bacteriology, while the second volume covers virology, mycology, and parasitology. This is obviously an important resource for clinical microbiologists, but it also makes a useful go-to reference for summary and facts needed for teaching the medical/clinical side of the field.

Daniel P. Haeusser
Originally published in Small Things Considered,
April 14, 2016.
Practical Handbook of Microbiology, 3rd Ed.
Emanuel Goldman and Lorrence H. Green (ed.). 2015. CRC Press, 1,055 p., $197.95 (hardback).

For those who may not get much use out of the mammoth two-volume reference above, but would appreciate a more compact volume that also extends past clinical application, this volume may be of interest. Like the work above, this handbook starts with a section of chapters that cover general microbiological practices and principles, including sterilization, antibiotics, identification and quantitation, and epidemiology. A particularly fascinating chapter on the “Business of Microbiology” ends the first section, with details on hospital management, health insurance, and government regulations (among others). The second section narrows focus to specific groups of organisms with greatest emphasis on bacteria. But it also includes chapters on viruses (including some on phage), fungi, parasites, and archaea. The lack of figures in this handbook makes it unsuitable for something like an undergraduate textbook, but as a supplementary resource for student or instructor it would be very beneficial in its comprehensive scope across general microbiology.

Daniel P. Haeusser
Originally published in Small Things Considered, April 14, 2016.
Application Deadlines

ASM Grant Writing Course. Senior-level graduate students, postdoctoral scientists, and early-career scientists are invited to apply for the ASM Grant Writing Course (formerly the ASM Kadner Institute). Sponsored by the ASM Committee on Graduate and Postdoctoral Education to meet the growing need for guidance and support on grant applications, the course will take place 12-14 August 2016 in Washington, D.C. The course will emphasize excellence in grantsmanship, and participants will receive in-person mentoring, real-time constructive feedback, and best practice strategies for composing effective grant proposals. ASM offers the Grant Writing Course with partial support from the ASM-NSF Leaders Inspiring Networks and Knowledge (LINK) Program and the Burroughs Wellcome Fund. For additional program details, such as costs and eligibility criteria, please visit http://bit.ly/asmgw16nl.

Deadline: 30 June 2016.

ASM Scientific Writing and Publishing Course. The ASM Committee on Graduate and Postdoctoral Education welcomes applications to the ASM Scientific Writing and Publishing Course, an effort that supports beginning researchers in understanding the writing, publishing, and review processes for scientific journals. Set for 12-14 August 2016 in Washington, D.C., the course is led by ASM members who have published widely, reviewed manuscripts, and served on the editorial boards of major journals. Program benefits include one-on-one feedback from facilitators, writing practice, and stimulating group discussions and interactions. The course is open to senior-level graduate students, postdoctoral fellows, and early-career scientists who are ready for an immersive and intensive writing experience. ASM offers the Scientific Writing and Publishing Course with partial support from the Burroughs Wellcome Fund. For additional program details, such as costs and eligibility criteria, please visit http://bit.ly/swpc16nl.

Deadline: 30 June 2016.

Undergraduate Faculty Research Initiative Fellowships. Early-career (and future) undergraduate STEM educators are encouraged to apply for a 2016 ASM-NSF LINK Undergraduate Faculty Research Initiative (UFRI) Fellowship. This nascent professional development resource trains STEM faculty to develop undergraduate research programs by initiating successful research partnerships. As part of the fellowship, LINK will provide travel subsides of up to $2,000 to (i) increase participation of undergraduate STEM educators at eight eligible ASM-sponsored research conferences, (ii) encourage networking and collaborations with potential research partners, and (iii) access resources and mentoring to advance undergraduate research programs. Fellowship applications are accepted on a rolling basis for each of the eight conferences. The deadline is 18 July to be considered for a UFRI fellowship for the ASM Conference on Beneficial Microbes (Seattle, WA).

WWW: http://www.asmlink.org/ufri

Turning Your Science into a Company. To guide beginning investigators in combining their research interests with entrepreneurial ventures, ASM offers its third annual “Turning Your Science into a Company,” a workshop on establishing scientific businesses. Join the program on 6–8 October 2016 in Washington, D.C., to and receive valuable tips, advice, and resources from successful principals of leading start-up and small scientific companies. The workshop features examples from the biotechnology industry and specifically targets advanced graduate students, postdoctoral fellows, and early-career scientists.

Turning Your Science into a Company is sponsored by the ASM Committee on Graduate and Postdoctoral Education.

Deadline: 20 August 2016.
ASM Meetings Calendar

31 July–3 August 2016.
ASM Conference on Streptococcal Genetics.
Washington, D.C.
http://conferences.asm.org/

4–7 August 2016.
2nd ASM Conference on Experimental Microbial Evolution.
Washington, D.C.
http://conferences.asm.org/

29 August–1 September 2016.
5th ASM Conference on Salmonella.
Potsdam, Germany.
http://conferences.asm.org/

9–12 September 2016.
6th ASM Conference on Beneficial Microbes.
Seattle, Wash.
http://conferences.asm.org/

ASM Conference on Infection and Cancer.
Washington, D.C.
http://conferences.asm.org/

11–14 December 2016.
ASM Conference on Antibacterial Development.
Washington, D.C.
http://conferences.asm.org/

6–8 February 2017.
ASM Biothreats Conference: Research, Response and Policy.
Washington, D.C.
http://conferences.asm.org/

33rd Clinical Virology Symposium.
Savannah, Ga.
http://conferences.asm.org/

1–5 June 2017.
ASM Microbe 2017.
New Orleans, La.

About the Calendar

The ASM Meetings Calendar is provided as a service to readers of Microbe. It includes annual meetings and conferences organized by the Society. Detailed information for these events is published in the ASM Meetings and Conferences insert, which appears bimonthly in the center of Microbe.

As an added benefit of membership in ASM, an online calendar of microbiology-related meetings hosted by ASM and by other organizations is available through the ASM website. Any organization may submit items for the online calendar provided that submissions are of obvious interest to microbiologists. ASM will not permit announcements to appear in the calendar when the subject matter and dates conflict with ASM meetings or workshops. The calendar is located at https://info.asm.org/index.php/meeting-and-event-calendar. All entries in the online calendar are limited to conference name, dates, location, website, and contact information (person, address, telephone, fax, and/or e-mail). When websites and e-mail addresses are provided, links to them will be established. Because of the volume of submissions received, ASM staff is unable to provide proofs or other confirmation of receipt of each listing. Submit items for the online calendar through the “Add a new event/deadline” link on the Meeting and Event Calendar page.

March 2017.
ASM Conference on Innovative Microbial Ecology for Mitigation of Antibiotic Resistance and Bacterial Diseases.
www.asm.org/conferences

March 2017.
ASM Conference on Mechanisms of Intercellular Cooperation and Competition.
www.asm.org/conferences

April 2017.
ASM Conference on Tuberculosis: Past, Present and Future.
www.asm.org/conferences

May 2017.
ASM Conference on Interplay of Viral and Bacterial Pathogens (ASM-ASV collaboration).
www.asm.org/conferences

September 2017.
2nd ASM Conference on Rapid Applied Microbial Next-Generation Sequencing and Bioinformatic Pipelines.
www.asm.org/conferences

October 2017.
6th ASM Conference on Cell-Cell Communication in Bacteria.
www.asm.org/conferences

November 2017.
ASM Conference on Vibrio2017: The Biology of Vibrios
www.asm.org/conferences

December 2017.
4th ASM Conference on Viral Manipulation of Nuclear Processes.
www.asm.org/conferences
Employment

POSITIONS AVAILABLE

Postdoctoral Positions in Enzymology and Microbiology

Postdoctoral positions are available for enzymologists and microbiologists in enzyme and pathway discovery as part of a new multidisciplinary Program Project (P01GM118303, Novel Strategies for the Discovery of Microbial Metabolic Pathways). We are especially interested in applicants with demonstrated expertise in microbial genetics or mechanistic enzymology. The Program Project has the goal of developing sequence/structure-based strategies for facilitating assignment of in vitro enzymatic and in vivo metabolic roles of widely conserved enzymes of unknown function discovered in genome projects, a crucial limitation in microbial genomic biology. The project integrates bioinformatics, genetics, and metabolomics, structural biology, and computation with enzymology. The components of the Program Project are located at the University of Illinois (enzymology and microbiology; J. E. Cronan and J. A. Gerlt), Albert Einstein College of Medicine (structural biology and ligand screening; S. C. Almo), and University of California, San Francisco (modeling, docking, pathway prediction; M. P. Jacobson, A. Sali, and B. K. Shoichet). Due to the collaborative and multidisciplinary environment, the Program Project provides an opportunity to receive training in several areas. To apply or request details, please send an e-mail to enzymes@igb.illinois.edu.

Postdoctoral Position—Microbiology and Sediment Bioremediation

A postdoctoral position is immediately available at the University of Minnesota to work on the bioremediation of contaminated estuarine sediment by the deposit-feeding polychaete Capitella teleta and its microbiome. The post-doctoral project will examine the relationship between this worm and its microbiome and quantify the importance of the microbiome for contaminant metabolism and sediment bioremediation. All applicants must have expertise in microbiology, microbial ecology, and biodegradation. The position is for 2

Employment Advertising

Microbe is published monthly and available to nearly 40,000 ASM members and institutional subscribers. Lead time for employment ads is about 3 weeks. Microbe is mailed around the 8th of the month of issue, but the delivery date is not guaranteed. Please consider delivery dates when setting application deadlines.

ASM does not accept classified advertisements that indicate a limitation, specification, or discrimination on the basis of race, religion, national origin, sex, mental or physical disability, age, or any other matters which may not be lawfully considered in making employment decisions. Employment notices that discriminate against microbiologists on the basis of a particular board certification or doctoral degree will not be accepted. Such advertisements will be rejected unless it can be established that the position by state or federal law or regulation requires a specific board certification or doctoral degree.

Classified

Classified ads must be typed, double spaced, with normal sentence capitalization (capital and lowercase letters). Microbe cannot accommodate requests for extra capitalization, boldface type, or other text or layout enhancements in classified ads.

Include the name and telephone and fax numbers of a contact person for questions about your ad copy. Incorrectly typed ads or ads with application deadlines earlier than the 15th of the publication month requested cannot be guaranteed placement in that issue.

Deadlines: Your ad must be received by the 1st of the month before the publication month to ensure timely publication (e.g., to appear in the September 2016 issue, your ad must be received by 1 August 2016).

Classified ads should be sent (with payment) to Walchli-Tauber Group, 2225 Old Emmorton Road, Suite 201, Bel Air, MD 21015, attn: Rhonda Beamer, tel. (443) 512-8899x106; fax, (443) 512-8909; e-mail, rhonda.beamer@wt-group.com.

Rates:

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Internet posting: All classified line advertising printed in Microbe also appears on the ASM website. Ads are posted to the website shortly before the issue mailing date and remain on the site for approximately 1 month. Hence, line ads placed for an issue of Microbe will be available to ASM website browsers around the beginning of the month and will overlap their print appearance in the magazine. All ads are replaced with the close of the next issue.

For display ad Internet posting costs, please contact Rhonda Beamer at the address given above.

Display

Display advertising closes the 1st of the month preceding publication. For specifications, rates, and deadlines for display ads, contact Rhonda Beamer at the address given above.
years, and includes a competitive salary and health insurance. Applications should include: (i) brief cover letter, (ii) curriculum vitae, (iii) a brief description of past research accomplishments and future research goals (under two pages), and (iv) the names and contact information for three references. All applicants need to apply online at http://www1.umn.edu/ohr/employment/ and click the link in the center of the page that corresponds to their situation. The Search Job ID# is 310096. Questions can be directed to Valery Forbes (veforbes@umn.edu) and Mike Sadowsky (sadowsky@umn.edu) with “Postdoc” in the subject line. The University of Minnesota is an Equal Opportunity Employer.

Endowed Chair in Biomedical Research as an Associate or Recent Full Professor of Virology

The Viral Information Institute is a new interdisciplinary research center at San Diego State University (SDSU) built upon the pioneering work in virology of a diverse group of collaborative faculty. The institute aims to understand the impact of viruses on human health and the environment through cutting-edge research. The Department of Biology at SDSU is recruiting an individual as either an associate professor or a full professor promoted to full within the last 5 years to be the Conrad Prebys Endowed Chair in Biomedical Research as part of the Viral Information Institute. The successful candidate will have a demonstrated record of research accomplishments and funding and will employ state-of-the-art approaches to study the role of viruses in the human microbiome. The candidate will also have a strong record in grant writing and extramural support, as well as a demonstrated capacity for collaborating, mentoring, and teaching. Applicants should apply via Interfolio at http://apply.interfolio.com/34893. Review of applications will begin 01 August 2016, and will continue until the position is filled. Incomplete applications are not guaranteed full consideration. For more information see: http://www.bio.sdsu.edu/jobs/. SDSU is a Title IX, equal opportunity employer.
Species have been recently reported to be mixotrophic, and there are likely many more utilizing a diverse range of feeding mechanisms. Some generate water currents using flagella or pseudopods to draw prey into the cell through a central groove called a sulcus. Others, including several *Protoperidinium* species, extend a modified pseudopod called a pallium to engulf and digest prey extracellularly. Pallia can expand up to 10 times the diameter of the dinoflagellate, allowing the consumption of large prey, including entire chains of diatoms. Lastly, others utilize a feeding tube-like structure called a peduncle to attach to prey and suck out their guts.

It can be difficult to study mixotrophy experimentally because both predator and prey can be quite small, making observation difficult. Another complication is that feeding might be induced only by particular conditions. To add to this, many dinoflagellate species have not been cultured. But despite these problems, some things are known about common feeding modes. Some species will only ingest prey when organic nutrients are limiting (e.g., *Ceratium furca* and *Prorocentrum minimum*), and addition of nutrients to medium inhibits their feeding. Others will feed even when nutrient conditions are replete (e.g. some *Dinophysis* species). An excellent test of whether a photosynthetic dinoflagellate is actually a mixotroph (barring visual evidence of it feasting on unfortunate prey) is to see whether it can grow in the dark. The growth rate of the dinoflagellate *Fragilidium subglobosum* increases at many different light intensities, and it can grow quickly in the dark when prey is provided.

It can also be challenging to determine whether heterotrophs are also photosynthetic, as in the case of *Dinophysis* species. It remains a mystery whether they use their own chloroplasts or those of their prey. While molecular evidence suggests that the chloroplasts of the dinoflagellate and of its prey, the ciliate *Mesodinium rubrum*, are identical in DNA sequence, microscopic evidence suggests that structurally, their chloroplasts are quite different. This example demonstrates how it is not always clear what an organism’s trophic strategy is. Many dinoflagellate species seem to exist on a continuum between being permanently photosynthetic, “borrowing” photosynthetic capabilities, and dispensing with photosynthesis altogether. But does borrowing photosynthetic capabilities count as mixotrophy? For example, marine animals such as corals, sea anemones, and clams supplement their food supply with carbon fixed by endosymbiotic photosynthetic dinoflagellates specifically recruited for this purpose. While this is an interesting example of a trophic expansion, mixotrophy typically refers to the “eating habits” of a single organism. However, as we increasingly realize just how interconnected different life forms are, this distinction becomes blurry.

With the seemingly endless diversity of dinoflagellates, it is not surprising that their ways of obtaining nourishment are equally varied. Thus, while we are left at the end of the day deciding between a burger out or a salad at home, the dinoflagellates have already decided: they’ll have both!

Rachel is a Ph.D. student in Andrew Allen’s lab at the Scripps Institution of Oceanography and the J. Craig Venter Institute in La Jolla, Calif., and Elie is a Medical Writer at Arbor Scientia in Carlsbad, Calif.