"Every speck of dust, drop of water, and grain of soil and each part of every plant and animal contain their own worlds of microbes."

"We desperately needed a book that climbs the big tree, branch by branch, written both for undergraduates and as a reference. Principles of Microbial Diversity is that book!"

— JO HANDELSMAN
Howard Hughes Medical Institute Professor, Frederick Phineas Rose Professor, Department of Molecular, Cellular and Developmental Biology, Yale University
Phenotype MicroArrays enable cells to be evaluated for thousands of phenotypes and under thousands of culture conditions in a simple, rapid, cost-effective manner. DNA Microarrays and Proteomic Technologies offer vast amounts of data, but often do not provide the complete information your research needs. Let Biolog help you reveal what other technologies can not see.
FEATURES
13 Federal Officials, Congress Seek Ways To Stimulate Antibiotic Development
Jeffrey L. Fox
Concerns over drug resistance are helping to spur policy proposals for fostering antibiotic development efforts

17 Quorum Sensing and Social Interactions during Infection
Eric J. G. Pollitt, Freya Harrison, and Stephen P. Diggle
Social evolution is an active area of research in microbiology, opening new approaches to understanding environmental adaptations, including virulence

23 Methane Fuels Deep-Sea, Rock-Hosted Ecosystem
Jeffrey J. Marlow
Symbiotic clumps of methanotrophic archaea and sulfate-reducing bacteria anaerobically oxidize about 80% of the methane released from sub-seafloor seeps

ANIMALCULES
2 TB or Not TB?
Bernard Dixon
The author’s ambiguous encounter with Mycobacterium tuberculosis 60 years ago is now an amusing but entirely trivial footnote to the continuing saga of a great killer

CURRENT TOPICS
2014 ICAAC
4 Sometimes Baffling Tickborne Microbial Illnesses Continue To Emerge
5 Host Gene Changes Affect How Bacteria Colonize Those Hosts
5 Several Strategies in Search for Agents To Treat MERS-CoV

RESEARCH ADVANCES
7 Progress in Efforts To Harness Yeast for Making Opioid Drugs
7 Microorganisms in Oceans May Arise Faster than Currents Disperse Them

NEW IN ASM JOURNALS
9 Engineered Chestnut Withstands Blight but Spares Fungi at Its Roots
10 Highlights from Recent ASM Journals

DEPARTMENTS
1 Letters
39 Reviews and Resources
41 Application Deadlines
42 Calendar
44 Small Things Considered
NEXT MONTH

After Horizontal Gene Transfer, Metabolic Pathways May Need Further Optimization
Joshua K. Michener and Christopher J. Marx
Effective use of a horizontally transferred pathway can require co-evolutionary changes in the host or pathway

Encouraging Microbiology Students To Think Like Scientists
Erica Suchman
Instead of urging students to memorize new materials, teach them to understand concepts fully and then put them to use

Climate Change Broadly Increases Infectious Disease Risks
Shannon Weiman
Climate change could prove damaging to individual plant and animal species, to complex ecosystems, and of course to humans

ASM NEWS

30 ASM Communications Committee Strategic Plan
30 ASM Speakers’ Bureau

SECTIONS

32 ASM Public Affairs
34 Education Board
35 International Affairs
35 Center for the History of Microbiology/ASM Archives
36 Obituaries

ASM LISTSERVS AND MICROBE_ALERT

Go to http://www.asm.org/index.php/forms/subscribe-to-listserv.html on the Web to sign up for ASM listservs, including the ASM Weekly Newsdigest. Subscribe to Microbe_Alert to receive the table of contents of the latest issue of Microbe when it becomes available online.
Start the school year off right with Free Teaching Resources!

Expert advice from the American Academy of Microbiology brought to your classroom.

Freely available: bit.ly/ASMFAQ

Topics include:
- Oil Spills
- West Nile Virus
- Influenza
- Adult Vaccination
- E.coli

Learn about microbiology career opportunities!

Invite an ASM speaker to your next Student Chapter meeting!

The Speakers’ Bureau currently features experts in areas such as biosafety, clinical microbiology and immunology, dairy, food safety, medical device, pharmaceuticals, and public health, and they want to talk to ASM students about their dynamic careers in microbiology.

Hear first-hand from speakers who have contributed to microbiome projects and biosecurity planning and chat with outbreak responders about curious cases!

If you are interested in hosting a live, recorded or video call presentation, visit http://bit.ly/ASMspeakers.
Letters

To Speciate or Not, That is the Question

We have observed in the microbiology community, in particular the clinical microbiology community, that there is often confusion regarding the correct use of the noun speciation and the verb “speciate.” The Oxford Dictionaries website (http://www.oxforddictionaries.com/us; accessed 14 September 2014) defines speciation as “the formation of new and distinct species in the course of evolution.” It has been our finding that speciation and speciate are frequently used to indicate that an organism was identified to the species level. When describing the specific identification of an organism, it would be more appropriate, indeed correct, to indicate that an organism was identified to the species level.

Rather than providing an anecdotal report of our experience, we sought to quantify this observation. To do this, we reviewed 100 randomly selected peer-reviewed articles that included the terms speciation and/or speciate published between January 2010 and July 2014 in the following clinical microbiology-related journals: Antimicrobial Agents and Chemotherapy, Clinical Microbiology Reviews, Infection and Immunity, and the Journal of Clinical Microbiology. Only 15 of the 100 publications unequivocally applied the terms speciation and/or speciate correctly when included in the main body of text. In 59 articles the terms were absent from the main body of text and only included in cited references. However, the majority of references containing these terms addressed a topic directly related to microorganism identification; thus, implying incorrect usage.

In conclusion, we have found that despite the frequency of their use in the scientific literature, the words speciation and speciate are often used incorrectly, and that the misuse of their intent is most notable in clinical microbiology-related journals. Therefore, we hope that through our letter we will promote their correct use in the scientific literature.

Lars F. Westblade
Children’s Healthcare of Atlanta
Emory University School of Medicine
Atlanta, Ga.

David H. Pincus
bioMérieux Inc.
Hazelwood, Mo.

MALDI-TOF Mass Spectrometry in Clinical Labs

I wish to respond to the excellent article by Scott A. Cunningham and Robin Patel of the Mayo Clinic entitled “MALDI-TOF Mass Spectrometry in a Clinical Lab Setting” in the August 2014 issue of Microbe (p. 328–333). This particular article addresses a very important issue in the clinical microbiology laboratory. It is written in clear, easy-to-understand laymen’s language with superior illustrations of the scientific principles involved. The article serves to completely eliminate fears and paranoia of the new technologies for laboratory workers who have been in the field for over 20 years and may feel threatened. The old adage is still very true. You don’t have to have a Ph.D in physics to use electricity.

Arthur P. Guruswamy
Consolidated Laboratories of the Commonwealth of Virginia, Richmond
TB or Not TB?

The author’s ambiguous encounter with Mycobacterium tuberculosis 60 years ago is now an amusing but entirely trivial footnote to the continuing saga of a great killer

Bernard Dixon

I felt vaguely unwell during some important exams at school, but attributed this to anxiety, having done very little work beforehand. Then, the following Saturday, just I was getting ready to go out dancing, I suddenly found myself coughing up warm, frothy blood—spurting, trickling, then gushing again. Very nasty. Hemoptysis is a horrible experience, one I shall never forget. That’s why, for me, one of the most vivid passages in English literature is H. G. Wells’s description in his autobiography of the occasion when the same thing happened to him.

First reactions. Bewilderment. Fear. Suspicion that the family doctor was incompetent when, after several phone calls from my parents, and two visits, he still advised me to suck ice cubes to deal with “a nose bleed.” He simply did not recognize the real source of the scarlet, bubbly fluid I was spewing up. Then, in the small hours of the night (after the doctor had thought again), my dislike of hospitals vanished magically in the ambulance. Hospitals now meant imminent safety. All of this happened six decades ago, yet the memories remain vividly clear. I remember the lovely sips of cold milk—all I was allowed to consume for the first two days. My dread of the next eruption after each brief hiatus. Well-meaning but unwelcome sounds of hymn-singing from along the corridor on Sunday morning. And resentment over that doctor. Most of all, I recall relief at being attended by medicos—which, however, faded quickly when I began to believe that they were puzzled too.

Gradually, things improved. Staunched by vitamin K, the deluge abated and I felt slightly better. Injections, examinations, pills, pokings. “It’s for what you’ve got,” said one nurse haughtily when I inquired what she was injecting into my bottom. X-ray plates were taken, peered at, and muttered over. “A slight shadow on the left upper lobe,” said the radiographer, “but we’re not sure. It’s a bit ambiguous.”

Definitive diagnosis proved awkward. Late on my third evening a nurse handed me a screw-capped bottle and demanded “a sputum sample in the morning.” I didn’t know what sputum was. Even when she told me, I had to confess that I did not have any. Not even a cough.

“Just do as you’re told, my lad.”

She returned at 6.00 AM. Sputum from the patient in bed number 12 was her last duty before going off for breakfast. “I’ll be back in five minutes,” she warned me, glowering at the empty bottle, “and I want to see it full.”

Noticing that she was very cross, I filled the receptacle with saliva. I did the same thing every morning for the required week, and watched the samples, handled like vials of a hazardous isotope, being packed off for the lab. They never did find the Mycobacterium tuberculosis they were looking for.

Bacilli or no, I was next moved to a very strange hospital. It was an all-male enclave in a decrepit old country house, run by monks who spent part of their time tending patients and part loping off to mass and vespers. They seemed happy and carefree. When Brother Richard tried out the sputum bottle routine on me, he wasn’t at all angry when I could not oblige. Like the rolls of fluff under the beds, it didn’t seem to matter.

There was just one occasion when I felt uneasy about the somewhat-less-than-24-hour medical cover. It arose from the constant inducements, in this bizarre outpost of Britain’s National Health Service, to eat gargantuan volumes of food. Particularly vital—and a matter of competition between patients—was the number of slices of bread one consumed on top of an ample supper. One evening, in boastful mien, I ordered eight, and ate them all. This provoked vigorous palpita-
tions an hour later, so I rang the bedside bed. But all the brothers were away chanting in the chapel. Symptoms, it transpired, were best indulged between the daily offices—and preferably at slack times in the liturgical year. Looking back, I can only assume that the hospital’s urge to make bedridden patients overeat reflected medical belief that TB was a disease closely associated with malnutrition.

Many of the “lay” staff were ex-patients who had simply stayed on. Some were mentally subnormal. Some were both. You never quite knew who were patients and who were orderlies. Fact and fantasy were inseparable, and the only contact with hard reality was the occasional visitation by doctors from a conventional hospital a few miles away. Gossip thrived, most of it originating with the elderly patients upstairs who had real, chronic pulmonary TB. “Are you for the knife?” I was asked on my first day by a furtive figure in a white coat. He grinned fiendishly when I said no. Later, he emerged as Joe, one of the local characters. The authorities had long since forgotten whether he was a patient, paid help or visitor. And very nice too, having the odd oddity about the place—but not to frighten news arrivals out of their wits.

Surgery was greatly feared along the old hands upstairs, and a topic of sorcerous talk among Joe and his mates. There was a belief that AP (artificial pneumothorax) and PP (pneumoperitoneum), far from being techniques for resting a diseased lung, were actually methods of punishment. I myself certainly doubted the motives of the very tall, austere monk who came late every night, waking people up to demand that they take sleeping tablets. “Pale pills for pale people,” he enunciated thinly as he glided from bed to bed.

Fortunately, I was soon moved to a conventional sanatorium. In the past, the monastery/hospital/madhouse had been occupied entirely by chest patients, but the supply of TB victims had fallen dramatically, thanks to streptomycin, mass radiography, and improved living conditions. Now the place was only half full, and many of the patients were like me with mediocre symptoms or no symptoms whatever. So it was turned over to orthopedic patients with genuine troubles, and the remaining chest cases were redeployed.

Looking back, it’s clear that the emptying of the madhouse was one of many indications that TB was retreating spectacularly. *Requiem for a Great Killer*, the title of a book by Harley Williams, published over a decade later, seemed to symbolize the triumph of medical science over one of the most venerable scourges of mankind. Although Williams was careful to warn readers that the final victory had not yet been won, there was no question of the confident mood of those times.

And today? The World Health Organization’s *Global Tuberculosis Report 2014* shows that nine million people developed the disease in 2013, and that 1.5 million of them died—including 360,000 victims who were HIV-positive. Publishing the report last October, the WHO announced that there were almost half a million more cases than previously estimated. Meanwhile, away from the gross statistics, we see developments such as the emergence of multiply resistant strains of *M. tuberculosis* and the identification of a previously unknown genotype that caused an outbreak in New York City in 2010.

I will never know whether, long ago, I had TB. It simply does not matter alongside the vast burdens of morbidity and mortality still caused by this physically tough, genetically versatile pathogen.
Sometimes Baffling Tickborne Microbial Illnesses Continue To Emerge

Shannon Weiman

Genomic analyses are enabling investigators to uncover novel tickborne bacterial diseases that were previously of unknown origin or were misdiagnosed as Lyme, which is caused by *Borrelia burgdorferi*. These flu-like illnesses include symptoms such as fever, headache, and fatigue, but are transmitted by specific species of ticks, typically in particular geographic regions, according to several researchers who spoke during the symposium “Tick-borne Infectious Diseases” at the 2014 InterScience Conference on Antimicrobial Agents and Chemotherapy, held in Washington, D.C., last September.

A particularly gruesome tickborne illness, called scalp eschar and neck lymphadenopathy after tick bite, emerged recently in Europe, according to Arantza Portillo of the Center for Biomedical Research in Rioja, Spain. Infected individuals characteristically experience necrotic skin lesions and swollen lymph nodes but lack the telltale rash of other rickettsioses or of Lyme disease, she says. Nearly all lesions appear on the head of affected individuals because the tick vector, *Dermacentor marginatus*, climbs to 1.5 meters above the ground to await its prey. Nearly all these ticks in Europe carry one or more of the causative species *Rickettsia rioja*, *R. raoultii*, and *R. slovaca*. “These pathogens have probably been circulating in Europe for a long time,” she says. A comparable disease, caused by *Rickettsia* spp. 364D, was recently reported in California.

Other gram-negative rickettsia species cause ehrlichiosis, another type of tick-borne disease. A new variety of ehrlichia was identified in 2009, and is found only in Minnesota and Wisconsin, according to Bobbi Pritt of Mayo Clinic in Rochester, Minn. That species is transmitted by *Ixodes scapularis* ticks, in contrast to other types of ehrlichia that are transmitted by *Amblyomma americanum* ticks, she says.

A Lyme-like disease, detected in the southern United States during the late 1980s, is called Southern-tick associated rash illness (STARI). Characterized by a more uniform rash than the bulls-eye rash that often occurs at the outset of Lyme disease, these early cases first appeared outside the geographic range of the primary tick vector of Lyme, *I. scapularis*. The Lyme spirochete “could not be isolated from human cases from the region, and the tick associated with the illness was *A. americanum*, which is not a vector for *B. burgdorferi*,” says Adriana Marques of the National Institute for Allergy and Infectious Diseases in Bethesda, Md. While the causal agent for STARI remains unknown, this illness appears to be more prevalent than Lyme in some areas, she warns. “*A. americanum* are the most abundant biting ticks in the southern United States, and their range extends all the way up to Maine.”

A tickborne agent, *Borrelia myamotoii*, found first in Japan in 1995, subsequently was found in several regions across Europe and North America, according to John Branda of Massachusetts General Hospital in Boston. This *Borrelia* species causes a severe acute illness that often requires infected individuals to be hospitalized, he says. It is transmitted by *Ixodes* ticks, and its
prevalence may be about 10% that of Lyme disease.

Shannon Weiman is a freelance writer in San Francisco, Calif.

2014 ICAAC
Host Gene Changes Affect How Bacteria Colonize Those Hosts

Shannon Weiman

Single-nucleotide polymorphisms (SNPs) in genes encoding immune receptors, signaling molecules, and other molecules not classically associated with immune responses can affect how well some bacterial pathogens or commensal species colonize individual hosts, according to several researchers who spoke during the 2014 Interscience Conference on Antimicrobials and Chemotherapy (ICAAC), held in Washington, D.C., last September. Exploring these associations, researchers hope to reveal mechanisms underlying disease pathology and other host-microbe interactions.

Some individuals are particularly susceptible to invasive pneumococcal disease (IPD), according to Anna Sangil Betriu of the Hospital Universitari Mutua Terrassa in Catalonia, Spain. Some 43 SNPs in 10 immune genes are linked to rare cases occurring in otherwise healthy individuals, “which may explain their susceptibility,” she says. Most prominent are minor alleles in the interleukin-1 receptor 1, which mediates innate inflammatory responses, and in two inhibitors of nuclear factor kB that regulate signaling of innate and adaptive responses. “If confirmed, these findings may help us to better understand the genesis of the illness, and to identify people at risk,” she says.

A single SNP in the gene for the leptin receptor (LEPR) appears to confer a threefold-greater susceptibility to infections by *Clostridium difficile*, according to Rajat Madan of the University of Virginia in Charlottesville. This same Q233R mutation in LEPR is also linked to susceptibility to *Entamoeba histolytica* infections, he notes.

Leptin, better known for its role in appetite and obesity, also influences gut integrity, microbiome composition, and inflammatory responses, Madan says. “Leptin is pro-inflammatory and augments the host defense during infections, presumably by enhancing immune responses.” The mutation impairs LEPR activation of signal transducer and activator of transcription 3 signaling, reducing mucosal chemokine production and neutrophil recruitment in mice, thus rendering them less able to clear *C. difficile* from the gut, he says. These findings “demonstrate a connection between metabolism and immunity.”

In other cases SNPs in host genes may influence the gut microbiota, thereby indirectly affecting susceptibility to pathogens or the likelihood of developing still other types of diseases, according to Jose A. Oteo of the Centro de Investigación Biomédica de La Rioja in Rioja, Spain. “Changes in gut microbiota composition may be responsible for a plethora of pathologies, including inflammatory bowel diseases, colon cancer, and obesity,” he says. For example, a SNP in the adrenomedullin gene (rs4910118), which decreases circulating levels of adrenomedullin, a peptide with antimicrobial properties, may dictate gut colonization by specific bacterial species. In female mice, knocking out this gene reduces the abundance of Bacteroidales and Clostridiales, while increasing Enterobacteriales, a bacterial order suggested to be implicated in high-fat-, diet-induced obesity, he says. He and his collaborators plan to examine whether humans with this SNP exhibit similar changes in microbiota and how this may influence their likelihood of developing various diseases. “This SNP is linked to cancer and high blood pressure,” he notes.

2014 ICAAC
Several Strategies in Search for Agents To Treat MERS-CoV

Shannon Weiman

Researchers are seeking to identify and develop agents that target the Middle East respiratory syndrome coronavirus...
MINITOPIC
Progress in Developing Disparate Vaccines

Regulatory officials and researchers announced progress with the development of several different kinds of vaccines, including:

- Officials of the Food and Drug Administration (FDA) in October approved Trumenba, a vaccine to prevent invasive meningococcal disease caused by Neisseria meningitidis serogroup B in individuals 10 through 25 years of age. The vaccine is made by Wyeth Pharmaceuticals Inc., a subsidiary of Pfizer Inc., in Philadelphia, Pa.
- A tetravalent vaccine directed against the Dengue virus proved effective in protecting children against the virus, and use of the vaccine led to fewer hospitalizations during a phase 3 clinical trial in five Latin American countries where dengue is endemic, according to Gustavo Horacio Dayan of Sanofi Pasteur in Swiftwater, Pa., and his collaborators. Details appeared 3 November 2014 in the New England Journal of Medicine (doi:10.1056/NEJMoai411037).
- An experimental nasal vaccine provides long-term protection for nonhuman primates against the deadly Ebola virus, according to Maria Crayle of the University of Texas at Austin and her collaborators. They reported recent findings during the annual meeting of the American Association of Pharmaceutical Scientists, held in San Diego, Calif., last November.
- A vaccine against the H1N1 influenza virus whose antigens were reformulated is “six times more active” than are conventional versions of the flu vaccine “in terms of hemagglutinin immunogenicity and in vivo protection,” according to Manuel Rosa-Calatrava of VirPath and Emmanuel Dejean of Calixar, both in Lyon, France.

(MERS-CoV), which kills about 30% of individuals that it infects. Promising leads are arising from target-based drug development approaches and also from efforts to repurpose drugs that already are approved for treating other conditions, according to several researchers who summarized recent progress during the 2014 Interscience Conference on Antimicrobial Agents and Chemotherapy, held in Washington, D.C., last September.

Several inhibitors of viral helicase, spike protein, and RNA synthesis enzymes look promising when tested in vitro against MERS-CoV, according to Jasper Chan of the University of Hong Kong in China. Several of them are also active against the closely related severe acute respiratory syndrome coronavirus (SARS-CoV). Additionally, monoclonal antibodies and plasma from patients recovering from MERS-CoV infections neutralize that virus, he says, noting that convalescent plasma is being evaluated in clinical trials in the Middle East.

The papain-like protease (PLpro) of MERS-CoV, which is essential for its replication, is another target for candidate drugs, according to Hyuan Lee and Michael Johnson of the University of Illinois, Chicago. Although many inhibitors of the SARS-CoV PLpro proved to be ineffective against the MERS-CoV PLpro, she and her colleague Hao Lei recently identified an inhibitor of both viral enzymes. “High-throughput screening of 25,000 compounds produced a dual inhibitor that acts as an allostERIC inhibitor against SARS-CoV PLpro and also acts as a competitive inhibitor against MERS-CoV PLpro,” says Lee. The compound also works in synergy with other lead inhibitors against SARS-CoV PLpro.

However, this target-based approach is painstaking, and it could take years before any of these promising viral inhibitors are approved as drugs, Chan cautions. An alternate and perhaps faster strategy involves testing currently approved antiviral agents, in hopes of repurposing some of them to treat MERS-CoV infections. Broad-spectrum agents, such as type-I interferons, or those used to treat SARS such as ribavirin and lopinavir appear promising, he says. Moreover, interferons synergize with ribavirin in vitro and in rhesus macaques, he says.

Yet another strategy casts a wider net and seeks to repurpose other types of drugs. For example, several cancer drugs, antidepressants, and hormone receptor modulators show anti-MERS activity, according to Lisa Hensley of the National Institute of Allergy and Infectious Diseases (NIAID) in Bethesda, Md. She says that Chris Coleman of the University of Maryland in Baltimore, Lisa Johansen of Zalicus in Cambridge, Mass., and their collaborators identified 6 SARS-CoV-specific inhibitors, 33 MERS-CoV-specific inhibitors, and 27 inhibitors of both viruses while screening a set of 290 drugs that were approved by the Food and Drug Administration (FDA) for other purposes. For example, chlorpromazine HCl, a neurotransmitter antagonist used to treat patients with schizophrenia, synergizes with various other drugs, Hensley says. Chan finds that mycophenolic acid, an immunosuppressant used to prevent rejection of transplanted organs, has anti-MERS-CoV activity, particularly in combination with Interferon β1b.

Between 2012 when MERS-CoV emerged and July 2014, this virus infected at least 837 people across 20 countries with a fatality rate of about 30%, according to the World Health Organization. SARS-CoV, which emerged in 2003, had a higher infectivity rate and caused about 8,000 cases worldwide that year and several dozen the next, but has not reappeared during the past decade. Its fatality rate was about 10%.
Progress in Efforts To Harness Yeast for Making Opioid Drugs

Carol Potera

*Saccharomyces cerevisiae*, more commonly known as baker’s yeast, is being developed as a means for producing opioid-based drugs, with the long-term goal of making those drugs from glucose instead of extracting them from poppy plants, according to Christina Smolke at Stanford University in Stanford, Calif., and her collaborators. They recently reported progress toward that goal after inserting genes from the poppy plant, *Papaver somniferum*, as well as other genes from *Pseudomonas putida* M10, which grows on poppy straw waste, into yeast cells—enabling them to produce opioids from intermediates that poppy plants make relatively early in that metabolic pathway and to do so with improved efficiency. Details appear October 2014 in *Nature Chemical Biology* (doi:10.1038/nchembio.1613).

Efforts to make such drugs in yeast prove to be painstaking, according to Smolke. In 2008, she and her collaborators engineered *S. cerevisiae* to produce salutaridine, a precursor of thebaine, a biochemical intermediate along the opioid biosynthetic pathway. “Salutaridine is just two enzymatic steps upstream of thebaine itself,” she says. In this more recent work, her team introduced genes into yeast that convert thebaine into hydrocodone, oxycodone, and hydromorphone. These semisynthetic opioids are considered safer and more effective than natural opiates extracted from poppy plants. However, now they are produced commercially by chemical rather than biosynthetic means.

In three enzyme-catalyzed steps, poppy plants convert thebaine to morphine. Smolke and her collaborators took the genes that encode those three enzymes—thebaine 6-O-demethylase (T6ODM), codeine O-demethylase (CODM), and codeinone reductase (COR)—and incorporated them into a yeast artificial chromosome (YAC). They also manipulated gene copy numbers and implemented other strategies to boost morphine yields. For example, they added two morphine dehydrogenase genes (*morA* and *morB*) from *P. putida* M10 onto the YAC to enhance the synthesis of both hydrocodone and hydromorphone. The modified yeast production strains churn out 51 milligrams/liter (mg/l) of hydrocodone, 70 mg/l oxycodone, and 1 mg/l of hydromorphone, she notes.

However, to be commercially competitive, these yields need to be improved by another 10- to 100-fold, according to Smolke. “We’re optimizing the [strains] to produce an integrated production system,” she says.

Efforts to develop a microbial fermentation system for making medically valuable opioids from glucose “moved one important step closer,” says John Dueber, a bioengineer at the University of California, Berkeley, regarding the efforts of Smolke and her collaborators to make opioids in yeast.

“Security measures will be required to prevent misuse,” he cautions.

Nonetheless, the availability of such a means for producing opioids could reduce our current reliance on farmed poppies, which are subject not only to plant diseases and climate disruptions but also to illicit trade practices. Australia and Tasmania grow much of the poppies used for legalized opioid production. Having engineered yeast as an alternative means for making these drugs will help to make the supply chain more robust, Smolke says.

Carol Potera is a freelance writer in Great Falls, Mont.
Efforts to understand how microorganisms in the gut affect the host continue to be part of the news. *Microbe’s* first set of examples for 2015 include:

- The microorganisms in the gastrointestinal tracts of humans are less diverse than those found in African apes, according to Andrew Moeller and Howard Ochman at the University of Texas at Austin and their collaborators. Details appeared 3 November 2014 in *Proceedings of the National Academy of Sciences* (doi:10.1073/pnas.1419136111).
- Delivering fecal transplant material via capsules proves both effective and safe as a means for treating persistent infections with *Clostridium difficile*, according to Ilan Youngster at Massachusetts General Hospital and Boston Children’s Hospital in Boston, Mass., and collaborators. Details appeared 5 November 2014 in the *Journal of the American Medical Association* (doi:10.1001/jama.2014.13875).
- Lipopolysaccharides and peptidoglycans from the gut microbiota stimulate specific inflammatory pathways in peripheral blood mononuclear cells that are correlated with alcohol craving, according to Philippe de Timary and Peter Stärkel of Université Catholique de Louvain in Belgium and their collaborators. Details appear November 1, 2014 in *Biological Psychiatry* (doi:10.1016/j.biopsych.2014.02.003).
- *Lactobacillus* species correlate, in the guts of mice, with mitigation of lupus symptoms, while Lachnospiraceae, a type of clostridium, correlate with worsening, according to Xin Luo of Virginia Tech in Blacksburg and collaborators. Details appeared 26 September 2014 in *Applied and Environmental Microbiology* (doi:10.1128/AEM.02676-14).
- Gut microorganisms produce γ-butyrobetaine from carnitine in red meat, giving rise to trimethylamine and trimethylamine-N-oxide, perhaps accounting for how meat accelerates atherosclerosis, according to Stanley Hazen, of Lerner Research Institute and the Miller Family Heart and Vascular Institute at Cleveland Clinic in Cleveland, Ohio, and his collaborators. Details appeared 24 October 2014 in *Frontiers in Microbiology* (doi:10.3389/fmicb.2014.00098).
- Disrupting the circadian clock in the host alters the rhythms and composition of the gut microbial community, helping to account for obesity and metabolic problems, according to Eran Elinav of the Weizmann Institute of Science in Rehovot, Israel. Details appeared 21 October 2014 in *Cell Metabolism* (doi:10.1016/j.cmet.2014.09.048).

*MINITOPIC*

**First Set of 2015 Gut Microbiota Studies**

Disrupting the circadian clock in the host alters the rhythms and composition of the gut microbial community, helping to account for obesity and metabolic problems, according to Eran Elinav of the Weizmann Institute of Science in Rehovot, Israel. Details appeared 21 October 2014 in *Cell Metabolism* (doi:10.1016/j.cmet.2014.09.048).

Gnotobiotic ferret L. Hellweger from Northeastern University in Boston, Mass., and his collaborators there and at the University of New South Wales in Sydney, Australia. Their conclusions are based on simulations of marine microbial geography, emphasizing genomic mutations embedded in models of ocean currents. Details appear in the September 12, 2014 *Science* (345: 1346–1349).

“*In a nutshell, microbes evolve faster than the ocean currents can disperse them,*” Hellweger says. “*Even in an environment that is often considered to be well-mixed, dispersal limitation can be substantial. This [finding] is in contrast to the common notion that microbes are not dispersal limited, or that ‘everything is everywhere.’”*

For their simulations of how microorganisms move and evolve within ocean environments, Hellweger and his collaborators modeled about 100,000 individual cells that divide and die, each with a 1-million-base-pair genome subject to mutations. Because those mutations are set as neutral and do not affect the growth or death of the microbes, any patterns can be attributed solely to neutral evolution and limits on dispersal. They sampled the population of cells at different times and locations, and then compared their DNA sequences using alignment tools. This analysis revealed emerging patterns, with microbial populations congregating in “provinces,” Hellweger says. Differences between those provinces grow gradually but then periodically collapse when populations coalesce.

“The evolution and distribution model [developed by] Hellweger and his collaborators is an excellent example of the growing use of simulation to explore potential hypotheses associated with biogeographic distribution of microbial assemblages,” says Jack A. Gilbert from the Argonne National Laboratory in Argonne, Ill., who helps to coordinate the Earth Microbiome Project. “They identify that microbial genomic evolution driven by local processes outweighs potential global mixing.

“The Earth Microbiome Project is characterizing the distribution of phylogenetic units and functional genotypes across the world ecosystems, and sees the same principle of distribution,” Gilbert continues. “Some . . . species, can be extremely well distributed, but strain-level variants of these species, some with extensive genotypic variance and, hence, functional ecology, are highly localized in time and space, exactly as predicted by this model.”

“We hope to develop models with better predictive power and reconnect modeling with contemporary observa-
tions, like environmental metatranscriptomics,” Hellweger says. One long-term goal is to model the transport of individual microbes in the oceans, taking into account their intracellular properties and behaviors, including genes, transcripts, proteins, and metabolism. “In this project,” he adds, “we took a natural first step—modeling individuals with whole genomes. Future work on microbe biogeography will have to consider environmental selection and neutral evolution and dispersal limitation.”

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

NEW IN ASM JOURNALS

Engineered Chestnut Withstands Blight but Spares Fungi at Its Roots

David C. Holzman

Chestnut trees that are genetically engineered to resist the pathogenic fungus Cryphonectria parasitica remain good hosts for symbiotic fungi that grow along their roots, according to William A. Powell of the SUNY College of Environmental Science and Forestry (ESF) in Syracuse, N.Y., and his collaborators. This sparing of benign fungi helps in overcoming a potentially important regulatory concern, keeping this transgenic variety of chestnut, Castanea dentata, on track for eventual widespread planting, he says. Details appeared 17 October 2014 in Applied and Environmental Microbiology (doi: 10.1128/AEM.02169-14).

“Before these trees can be used for a restoration program, they must be tested and then reviewed by three federal agencies—the U.S. Department of Agriculture, the Environmental Protection Agency, and the U.S. Food and Drug Administration,” Powell continues. “The American chestnut tree was one of the most abundant and important keystone tree species in the eastern forests of the U.S. Between three and four billion of these trees were lost to the exotic pathogen C. parasitica that was introduced into this country around 1900.” The American chestnut was highly valued by the lumber industry for its fast growth and rot resistance. The wood was also used widely for making musical instruments and furniture because it is both strong and lightweight.

The transgenic variety that Powell and his collaborators are testing is the first American chestnut tree variety that is specifically engineered to withstand that fungal blight. Its transgene from wheat produces an oxalic acid-degrading enzyme, oxalate oxidase, that targets C. parasitica. One potential complication is that nonpathogenic mycorrhizal fungi that live in symbiosis with the roots of trees, including chestnuts, could be an unintended target of that enzyme. Indeed, one important task of such fungi is to produce oxalic acid, presumably to enhance the uptake of minerals by causing biogeochemical weathering of minerals from rock.

“We compared root colonization among the transgenic American chestnut trees, wild-type American chestnut trees, chestnut trees produced by traditional hybrid breeding, and other tree species typically found near chestnuts in the wild,” Powell says. “The major relevant result from this work is that the Darling 4 transgenic American chestnut does not differ in ectomycorrhizal fungal associations from the wild-type chestnut.” These results hold true for trees grown in both greenhouse and field settings.

These findings address important questions “that will be required for regulatory approval in order to release transgenic chestnut into the wild,” says Ronald Sederoff of North Carolina State University in Raleigh. Unlike many other developments involving agricultural biotechnology, the “restoration of chestnut is not market driven,” he adds. “It has a primary objective as a social benefit, particularly to the environment and to rural communities. This difference should make the acceptance of a [transgenic] chestnut different from most previous agricultural crops.”

David C. Holzman is the Microbe Journal Highlights Editor.
NEW IN ASM JOURNALS

New Method Rapidly Determines Species and Susceptibility in Bacterial Infections

Overprescription of antibiotics is selecting for antibiotic resistance, contributing to one of the great medical problems of our time. Diagnosis of bacterial infections, and determination of antibiotic susceptibility profiles are slow and tedious, and consequently, patients may frequently receive antibiotics to which their particular infection is resistant. Now Mats Nilsson and Dan I. Andersson of Uppsala University in Sweden and collaborators have developed a general method to rapidly identify culprit bacteria species and determine their antibiotic susceptibility profiles. An initial, short cultivation step to be conducted both in the absence, and in the presence of different antibiotics is combined with a sensitive species-specific padlock probe detection of the bacterial target DNA, to determine whether the bacteria are growing or not, to indicate resistance versus susceptibility. In a proof-of-concept for urinary tract infections, they applied the method to determine the susceptibility profile of *Escherichia coli* for two drugs. Accuracy was 100%; duration, just 3.5 hours. That, the investigators write, would minimize the need for prescribing broad-spectrum antibiotics due to unknown resistance profiles of the treated infection.


Compounds Targeting DNA Packaging Enzymes Show Promise against Malaria Parasite

Malaria afflicts around 200 million people annually, killing more than 600,000, mostly in Africa, according to the Centers for Disease Control and Prevention. Now Nicholas A. Malmquist of the Pasteur Institute, Paris, France, et al. show that compounds derived from inhibitors of the histone-modifying methyltransferase enzymes kill malaria parasites in culture, as rapidly as the fastest-killing antimalarials available. They show further that these compounds are highly effective against multidrug resistant field isolates from Cambodia, and clinical isolates of the two most prevalent species of human malaria, *Plasmodium falciparum* and *P. vivax*. Furthermore, the compounds kill the malaria parasites specifically, that is, while remaining harmless to animal models and to their microbiomes. Additionally, they kill the parasites in both the form they take in mosquitos, and in that in which they inhabit mammalian hosts.

“All this suggests that this compound series can be developed into new antimalarials effective at both killing and reducing transmission of the relevant parasites currently threatening people in endemic regions,” says Malmquist.


Some Flu Viruses Potentially More Dangerous Than Others

Certain subtypes of avian influenza viruses have the potential to cause more severe disease in humans than do others. Jeffery K. Taubenberger of the National Institute of Allergy and Infectious Disease et al. show that viruses expressing the avian H1, H6, H7, H10, or H15 hemagglutinins led to rapid weight loss and fatal pneumonia infections in mice and caused more cell damage in normal human lung cells than did avian influenza viruses with other hemagglutinin subtypes. Conversely, mice infected with H2, H3, H5, H9, H11, H13, H14, and H16-expressing viruses suffered only mild weight loss, with no significant disease. The team showed similar results using hemagglutinins from two 2013 H7N9 flu viruses from outbreaks in China. These results suggest that hemagglutinins may not require immune cells to trigger cell damage, but instead may cause apoptosis or other molecular processes that could lead to fatalities, says Taubenberger.


Restrooms: Not as Unhealthy as You Might Think

Microbial succession in a sterilized restroom begins with bacteria from the gut and the vagina, and is followed shortly by skin microbes. “We

Downloaded from www.asmscience.org by
On: Mon, 03 Dec 2018 09:26:31
IP:  54.70.40.11
hypothesized that while enteric bacteria would be dispersed rapidly due to toilet flushing, they would not survive long, as most are not good competitors in cold, dry, oxygen-rich environments,” says coauthor Jack A. Gilbert of San Diego State University in California. Instead, as expected, skin microbes took over. Moreover, communities associated with each surface became increasingly similar in species and abundance within five hours of sterilization, and remaining stable for the remainder of eight weeks’ sampling. Ultimately, skin and outdoor-associated taxa comprised 68–98% of cultured communities, with fecal taxa representing just 0–15% of these. Outdoor-associated taxa predominated prior to sterilization, and long-term poststerilization, suggesting that long-term, human bacteria must be dispersed in restrooms in order to be maintained. Pathogens were not abundant, and methicillin-resistant Staphylococcus aureus was “Very rare,” says Gilbert.

Toilet seat samples, alone, clustered according to restroom gender, with Lactobacillus and Anaerococcus—vaginal flora—dominating women’s-room toilet seats, while the gut-associated Roseburia and Blautia were more copious on toilet seats in men’s rooms.


Sleeping Sickness: Research Suggests Potential Novel Intervention Strategy

Approximately 60 million people live at risk for sleeping sickness, caused by trypanosomes, notably Trypanosoma brucei, while livestock infections, which cause wasting disease, account for significant economic hardship in some of the most impoverished regions of the planet. Treatments are toxic, and increasingly ineffective. Thus, new perspectives are needed on trypanosome biology, transmission, and pathogenesis, in order to develop novel intervention strategies. Trypanosomes are capable of group-level behavior, but mechanisms governing T. brucei social motility have been unknown. Now Kent L. Hill and colleagues of the University of California, Los Angeles report that a subset of receptor-type adenylate cyclases in the trypanosome flagellum regulate social motility. RNAi-mediated knockdown of adenylate cyclase 6 (AC6) or dual knockdown of AC1 and AC2 causes a hypersocial phenotype but has no discernable effect on individual cells in suspension culture. Mutation of the AC6 catalytic domain phenocopies AC6 knockdown, demonstrating loss of adenylate cyclase activity is responsible for the phenotype. Notably, knockdown of other ACs did not affect social motility, indicating segregation of AC functions. “These studies reveal interesting parallels in systems that control social behavior in trypanosomes and bacteria, and provide insight into a feature of parasite biology that may be exploited for novel intervention strategies,” the investigators write.

CALL FOR ABSTRACTS - DUE JANUARY 27, 2015

Bring the History of Microbiology to Life

Submit an Abstract for the History of Microbiology Poster Session at ASM2015

Share your passion for the history of microbiology by submitting an abstract for the 2nd Annual History of Microbiology Poster Session:

- Explore and analyze an event, discovery or technique!
- Correct a misconception!
- Present new biographical information with a fresh, compelling analysis!

Last year’s session included 20+ posters on topics ranging from the impact of the Petri dish on microbiology all the way up to the impact of Apollo-Era microbiology on human space flights.

History of Microbiology Poster Session
Sponsored by the Center for the History of Microbiology/ASM Archives at the asm2015 General Meeting | May 30 - June 2, 2015 | New Orleans, LA

For Questions, Contact:
jkarr@asmusa.org or archives@asmusa.org
Deadline for Abstracts: January 27, 2015

Abstract Submission Procedures:
asm.org/choma-history-posters

---

One Health:
People, Animals, and the Environment

Editors: Ronald M. Atlas, University of Louisville; Stanley Maloy, San Diego State University

In One Health: People, Animals, and the Environment, editors Ron Atlas and Stanley Maloy have compiled 20 chapters written by interdisciplinary experts that present core concepts, compelling evidence, successful applications, and the remaining challenges of One Health approaches to thwarting the threat of emerging infectious disease. This book is a valuable resource for physicians, veterinarians, environmental scientists, microbiologists, public health workers and policy makers, and others who want to understand the interdependence of human, animal, and ecosystem health.

“This book provides a comprehensive overview of how the One Health concept needs to bring together human and veterinary and scientific communities to understand the events that underlie the emergence of new infectious challenges. Should be mandatory reading for anyone interested in zoonotic diseases and its quite the collection of international perspectives.”

—Christopher A. Hunter, Professor and Chair, Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania

When you buy books from the ASM Press, you support the society that supports the science of microbiology

WEB: www.ASMscience.org • PHONE: 703-661-1503 • EMAIL: books@asmusa.org
Federal Officials, Congress Seek Ways To Stimulate Antibiotic Development

Concerns over drug resistance are helping to spur policy proposals for fostering antibiotic development efforts

Jeffrey L. Fox

The President’s Council of Advisors on Science and Technology (PCAST) last September released a report, “Combating Antibiotic Resistance,” along with a strategy for dealing with this public health problem. In addition, the White House issued an Executive Order emphasizing the importance of addressing this challenge (Microbe, November 2014, p. 432). “Even with improved stewardship and surveillance, it is critical to develop new antibiotics, diagnostics, vaccines, and other interventions at a rate that outpaces the emergence of resistant microbes,” PCAST members noted. “A robust antibiotic pipeline is essential for creating new antibiotics to replace those being steadily lost to antibiotic resistance. Establishing this pipeline and successfully addressing the rise in antibiotic resistant bacteria will require coordination across governmental, academic, health-related, agricultural, and private sectors.”

That same week, the House of Representatives Subcommittee on Health, chaired by Rep. Joe Pitts (R-PA), convened a hearing, “Examining Ways to Combat Antibiotic Resistance and Foster New Drug Development,” part of a broader effort called “21st Century Cures.” The comments that follow were part of the public testimony presented during that September 2014 hearing. The Health Subcommittee is part of the House Committee on Energy and Commerce, chaired by Rep. Fred Upton (R-MI).

Economic Barriers Discouraging Antibiotic Development

“If we lose antibiotics as a drug class, the social cost may be more than a trillion dollars, shaving several years off life expectancy and making many modern medical procedures either impossible or much more dangerous,” said Kevin Outterson of Boston University School of Law in Boston, Mass. In terms used by economists, he added, “the net present value (NPV) of antibiotic investments [is] too low, especially compared with other investment opportunities within drug companies.”

The economic challenges for those developing antibiotics were analyzed in a report, “Analytical Framework for Examining the Value of Antibacterial Products,” that was prepared for the U.S. Department of Health and Human Services and released in April 2014, said Outterson, one of several experts who prepared that report. As a premise, the report “set a benchmark target of a NPV equal to or exceeding $100 million, which is a conservative target for a new antibiotic drug,” he said.

The analysis then examined the payback from developing six hypothetical drugs for treating different types of infections. “The results are striking,” Outterson said. “In no case did any of the six antibiotic drugs yield a NPV close to the benchmark $100 million. Put simply, society will benefit greatly from preventing or treating these conditions, but companies are not financially rewarded for bringing these products to market,

SUMMARY

➤ The White House and Congress are considering policy reforms that could stimulate antibiotic development.
➤ Recent analyses indicate that the return on investment for antibiotic development falls short of economic benchmarks, indicating important incentives are missing for this class of drugs.
➤ Recent laws as well as pending proposals seek to ease regulatory burdens on companies developing and clinically testing antibiotics.
➤ Improved diagnostics, particularly procedures for measuring antibiotic susceptibilities, would lead to better use of such drugs clinically and could also help toward encouraging their development.
and the U.S. health care system is not rewarded for preventing these infections through other means, such as vaccination, better diagnostics, or infection control.”

The 2013 GAIN Act Mandates FDA To Streamline Antibiotic Development

“Provisions in a law passed a little over two years ago, commonly known as the Generating Antibiotics Incentives Now Act, or the GAIN Act, are helping to stimulate the development of new antibotics,” said Janet Woodcock, Director of the Center for Drug Evaluation and Research (CDER) of the Food and Drug Administration (FDA). Under GAIN, antibacterial or antifungal drugs intended to treat serious or life-threatening infections can be designated as Qualified Infectious Disease Products (QIDPs).

As part of its QIDP designation, a candidate drug receives priority review and is eligible for fast-track designation. At approval, a product with QIDP designation may be eligible for an additional five years of marketing exclusivity, in addition to certain existing exclusivity periods under the Federal Food, Drug, and Cosmetic Act. To date, FDA has granted 59 QIDP designations for 39 different unique molecules.

“FDA is working hard to streamline requirements for clinical trials for studying new antibacterial drugs, and the provisions of the GAIN Act are being actively implemented,” Woodcock continued. “But more is needed. There are still significant economic and scientific challenges in the development of new antibacterial drugs that need to be addressed. Additional financial incentives, as well as new approaches to reducing the costs of studying antibacterial drugs, such as common clinical trial protocols, could provide other important means to stimulate antibacterial drug development.”

To help drive this effort, CDER assembled an Antibacterial Drug Development Task Force (Task Force), a group of expert scientists and clinicians from within FDA, to consider opportunities to help facilitate antibacterial drug development, according to Woodcock. FDA also has an agency-wide Task Force on Antimicrobial Resistance, which assures coordination of FDA activities across multiple product areas.

“As part of our Task Force’s collaborative efforts, FDA is working closely with the National Institutes of Health (NIH) to further advance the development of new antibacterial drugs,” Woodcock said. “In July 2014, we jointly hosted a two-day Public Workshop to identify strategies for promoting clinical trials for antibacterial drugs and encouraging partnerships to accelerate their development.

“FDA believes it is necessary to consider new mechanisms for encouraging the development of new antibacterial drugs to address unmet medical needs in the treatment of serious and life-threatening bacterial infections,” Woodcock noted. “One option is the establishment of a new Limited Population Antibacterial Drug (LPAD) program. . . . Drugs approved using an LPAD pathway would be based on more streamlined development programs that establish that the drug is safe and effective in a limited population of patients with serious or life-threatening infections and unmet medical needs.”

Other Legislative and Policy Proposals for Stimulating Antibiotic Development

“We find 38 antibiotics in phase 1 through 3 clinical trials, including five in advanced development with the potential to address gram-negative pathogens, the most pressing medical need,” said Allan Coukell, Director of Drugs and Medical Devices for the Pew Charitable Trusts in Washington, D.C. “This analysis is somewhat encouraging until one considers that the general rule for drug development is that 80% of products that enter clinical testing will fail for reasons of toxicity or inadequate efficacy.”

“Some of the most dangerous pathogens are to date occurring in relatively small numbers of patients, making it difficult or impossible to populate traditional, large-scale clinical trials,” said Barbara E. Murray of the University of Texas Health Science Center at Houston Medical School, who was the 2014 president of the Infectious Diseases Society of America. “It is important to develop drugs to treat infections caused by these deadly pathogens before they infect larger numbers of people. However, when a pathogen is resistant to all approved antibiotics, there is no effective antibiotic against which to compare the new antibiotic, which is the standard procedure for traditional clinical trials.”

“In its 2012 report, PCAST recommended an approval pathway for drugs for use in a limited population of patients with few or no other treatment options,” Coukell said. “This approach,
Challenges Faced in Bringing New Antibiotics into Use

Although FDA officials are adjusting their approach to evaluating antibiotics, plenty of challenges remain for this class of drugs, according to critics. Here, in brief, are how two new antibiotics have fared under regulatory review.

Achaogen’s lead candidate drug Plazomicin is being evaluated in a phase 3 clinical trial that uses a “superiority” design intended to demonstrate a reduced number of deaths among patients treated with plazomicin-based therapy as compared to the best available antibiotic care,” said Kenneth J. Hillan, who is chief executive officer of Achaogen, Inc. of South San Francisco, Calif., who spoke during the September 2014 hearing. “The trial design was agreed upon through the Special Protocol Assessment process, which is intended to provide assurance to sponsors that the trial design will be sufficient for market approval of the drug. Plazomicin also was granted Fast Track Designation, allowing frequent interaction with the agency throughout the planning process. We found our interaction with the FDA to be extremely collaborative and believe this serves as a model for how the FDA can facilitate development of antibiotics in a setting of urgent unmet medical need.”

Sirturo is a new antimycobacterial drug indicated as part of combination therapy in adults with pulmonary multi-drug resistant tuberculosis, or MDR-TB,” said Adrian Thomas, vice president of Global Market Access and Public Health at Janssen, the pharmaceutical companies of Johnson & Johnson in New Brunswick, N.J. “It is the first new medicine for TB with a new mechanism of action to be developed in more than 40 years, and is the first new drug specifically indicated to treat a drug-resistant form of tuberculosis.

“In keeping with the special requirements FDA and other regulatory agencies have set for Sirturo, our company’s post-marketing commitments are substantial,” Thomas said. “They include a lengthy phase 3 research program; a pediatric formulation and first-ever randomized, open label, controlled clinical study in a pediatric MDR-TB population; and a 5-year prospective study to characterize the acquisition of resistance to this new drug. . . . We estimate that approximately half of all investments necessary to develop and support Sirturo, amounting to several hundreds of millions of dollars, will be required after the point of U.S. regulatory approval in December 2012.

“Our experiences with Sirturo—today and since its discovery in our labs more than a decade ago—illustrate just some of the challenges associated with the development and introduction of new antibiotics,” Thomas continued. “These challenges help to explain why the overall state of antibiotics R&D is deficient relative to the need. They also point us to potential policy options for overcoming and counterbalancing current risks specific to antibiotics development.”

when applied to antibiotics, is referred to as a limited population antibacterial drug (LPAD) pathway. It would permit the FDA to approve new antibiotics for specific, limited populations of patients with unmet medical needs, such as those with highly resistant infections. The risk-benefit assessments for these individuals with limited treatment options would be different than for patients with susceptible infections, and the drugs may be approved for use based on smaller data sets. However, it is essential that this pathway be accompanied by strong labeling provisions to ensure health care providers are aware of the limitations of the data underlying the products’ approval.

“In December 2013, Representatives Phil Gingrey (R-GA) and Gene Green (D-TX), champions of GAIN, introduced the bipartisan Antibiotic Development to Advance Patient Treatment (ADAPT) Act, which would create an LPAD approval pathway for antibiotics filling an unmet medical need,” Coukell continued. “In addition, ADAPT would give FDA the authority to review promotional materials before a drug developer could use them for marketing, and would mandate retrospective evaluation to assess whether drugs approved through this pathway were prescribed as intended. . . . By allowing drug developers to rely on smaller datasets, and clarifying FDA’s authority to tolerate a higher level of uncertainty for these drugs when making a risk-benefit calculation, ADAPT would make the clinical trials more feasible than the larger clinical trials that companies now have to conduct in order to get a broader indication.”

Other policy proposals are being put forward to encourage companies to develop antibiotics, according to Adrian Thomas, vice president of Global Market Access and Public Health at Janssen, the pharmaceutical companies of Johnson & Johnson in New Brunswick, N.J. For example, the “Transferable Market Exclusivity (TME) is a policy incentive that was first proposed in 2003 by Duke University professor and researcher Henry Grabowski,” Thomas said. “TME is a pull-based incentive that affords companies a defined period
of market exclusivity that can be applied to any compound, thus facilitating R&D spending on a different ‘socially desirable’ but unprofitable medicines.

“Because the opportunity for commercial return on any new antibiotic product itself is so sharply limited, and because the spectrum of innovators required for antibiotics R&D today is so diverse, it is the transferable nature of the market exclusivity period made possible under TME—from one innovator to another, one product to another—that gives this model its unique strength as an innovation driver,” Thomas continued. “In addition to providing a meaningful incentive to innovators, TME decouples the investment toward development of an antibiotic from the market success of the antibiotic. This decoupling can help to mitigate any tensions between investment recovery and antibiotic stewardship post-market. We believe that TME can be structured in policy to maximize its public health advantages and to minimize downside risks, including risks to generic manufacturers.”

Role of Diagnostics when Testing or Administering Antibiotics

Generally, physicians rely on antimicrobial susceptibility test (AST) devices, which provide information about whether a bacterium is either susceptible or resistant to an antibacterial drug, said FDA’s Woodcock. “We need a better, more modern and streamlined administrative process to help AST device manufacturers incorporate up-to-date and comprehensive breakpoint information in their devices more quickly, in order to get this information to health care providers sooner for the care of patients. To address the problems with the current scheme for updating breakpoints, FDA needs to take breakpoints out of the drug product label and utilize more rapid, electronic means of communicating this information.”

“The lack of rapid diagnostic tests to quickly identify patients infected with certain pathogens who may be eligible for antibiotic or antifungal clinical trials early enough to improve their outcomes and to avoid enrolling patients only to find out 24 – 48 hours later that they are not eligible, adds markedly to the overall cost of the trial without gaining useful efficacy information,” Murray said. “IDSA recommends increased investment in diagnostics research, regulatory approval pathways for needed diagnostics, strengthening diagnostics reimbursement and supporting outcomes research to demonstrate the impact of diagnostics on patient care.”

“In an ideal world, rapid diagnostic testing would allow bacterial identification and antibiotic susceptibility to be determined at the point of patient care to enable health care professionals to decide on the most appropriate antibiotic as quickly as possible,” said Kenneth J. Hillan, who is chief executive officer of Achaogen, Inc. of South San Francisco, Calif. “Diagnostic tests can also be used to monitor drug exposure to individualize dosing for each patient, which has been shown to improve outcomes. We believe the federal government should be providing significant support and incentives to companies and innovators of rapid and cost-effective diagnostics that will advance antibiotic stewardship and clinical care.

“There is a need for an expedited approach to diagnostic development to keep pace with the changes in technology,” Hillan continued. “We need regulations that support a more flexible approach under a risk-based assessment that considers at its core, the overall benefit risk for patients. The regulations should provide the FDA with the flexibility to customize the required analytical studies for each assay at the time of NDA filing, as well as the data and testing related to quality systems, manufacturing, software testing and documentation, so that they support the safe and effective use of the drug.”

“The ADAPT Act contains important provisions designed to ensure that susceptibility test interpretive criteria, commonly referred to as breakpoints, for antimicrobial drugs are regularly updated in a timely fashion, and that updated breakpoints are made publicly available via FDA’s website,” Murray pointed out. “A breakpoint provides information that helps to predict whether a patient infected with a specific pathogen will have a good clinical response to standard doses of a drug. Given the ongoing development of drug resistance, it is critical that breakpoints be regularly updated to provide physicians with accurate information to guide the optimal use of drugs in patients.”

Jeffrey L. Fox is the Microbe Current Topics and Features Editor.
Quorum Sensing and Social Interactions during Infection

Social evolution is an active area of research in microbiology, opening new approaches to understanding environmental adaptations, including virulence

Eric J. G. Pollitt, Freya Harrison, and Stephen P. Diggle

Bacteria engage in diverse communal behaviors, such as forming biofilms, exchanging DNA, coordinating the production and release of enzymes and toxins, and moving in swarms. Scientists working in the branch of evolutionary biology called social evolution recently began to ask why individual cells interact in these ways.

The ideas underlying social evolutionary theory derive from studies of helping behavior, group formation, and reproductive cooperation in birds, mammals, and social insects such as bees and ants. A major historic focus of this work was to explain how these behaviors arose and what forces maintain them. Explaining why cooperation exists is problematic because many such behaviors appear to be costly. For example, the immediate effect of forgoing reproduction to help take care of another individual’s offspring, joining in a fight to support another individual, or sharing food reduces the fitness of the actor performing the behavior but increases the fitness of the recipient(s). Charles Darwin alluded to this problem, noting how insect drones give up reproducing for the benefit of their hive queen when it would seem to be more directly beneficial for them to breed themselves.

However, during the 1960s, William Hamilton of Imperial College in London, United Kingdom (UK), showed that individually costly behaviors could be deemed successful in evolutionary terms if they benefitted close kin. He termed this “inclusive fitness,” distinguishing it from fitness benefits that are purely “direct,” which only accrue to the actor. Inclusive fitness includes benefits that are direct but also ones which are “indirect,” which accrue to others who share the same genetic predisposition to altruism. Inclusive fitness is more commonly referred to as “kin selection” and forms the basis of the study of social evolution.

Social evolution has become an active area of research in microbiology for a number of reasons. First, microbes display many apparently social or cooperative behaviors, and many of these behaviors seem to be crucial for those microbes to survive in their ecological niches or to become successful pathogens. Biofilms are a prime example, as they allow microorganisms to colonize surfaces despite abrasion or flow, and they help to protect the microorganisms against desiccation, antimicrobial compounds, and host immune systems. Social evolution research also complements mechanistic studies—for example, by presenting problems for which there must be mechanistic solutions. Further, environmental conditions can be experimentally manipulated to test evolutionary theories that apply to microorganisms. Finally, rapid generation times allow for evolution experiments involving microbes to be run over many generations, with a high level of experimental control.

Individual microbial cells may interact in a variety of ways, including through quorum sensing (QS), coordinating production of nutrient-scavenging molecules, and forming multicellular biofilms.

Understanding whether a behavior is social can help us understand how bacterial populations interact within infected hosts, explaining some kinds of clinical observations as well as how virulence evolves.

Despite interest in this issue, experimental tests of whether bacterial behaviors such as QS are truly social are scarce.

From both mechanistic and evolutionary standpoints, QS is a social behavior.

Mutants that are defective in QS can be isolated from sites of infection, despite QS being important for virulence.
What Is Social Behavior among Microbes?

A social behavior is one that affects individuals other than, or in addition to, the individual performing that behavior. Behaviors are classified into four main types based on whether they confer a cost or a benefit to the actor and the recipient: these are mutual benefits, altruism (both forms of cooperation), selfishness, and spite (Fig. 1A), according to Stuart West, now at the University of Oxford in the UK, and his collaborators.

A basic and well-described form of cooperation in bacteria is the secretion of proteins and lower-molecular-weight compounds that enable cells to scavenge essential nutrients and resources from the environment. Such “public goods” include proteolytic enzymes, toxins, and iron-scavenging siderophores, which can be important virulence factors when the environment is an infected host. Producing these molecules is costly for individual cells. However, the benefits of their activities accrue both to producer and neighboring cells, regardless of whether the neighbors themselves make the costly exoproducts.

Thus, cells that cease to make the public good can avoid the production cost, but still benefit from the nutrients those compounds release through their interaction with the environment. They gain an increase in benefit in mixed populations with cooperators.

(A) Social interactions. Social interactions are divided into 4 groups based on whether they benefit or are costly to the initiator and/or recipient of the behaviour. (B) Social cheating. Bacterial cells act as cooperators when secreting compounds (e.g. enzymes) and this imposes a fitness cost. Cheater cells do not secrete these compounds but can benefit from the nutrients those compounds release through their interaction with the environment. They gain an increase in benefit in mixed populations with cooperators.
determining whether cooperators or cheats win out over evolutionary time. When cooperative acts are targeted towards relatives that also carry cooperative genotypes, cheats cannot easily gain a foothold. The spatial structuring of populations that enables such cooperative acts typically depends on limited dispersal of individuals or aggregates forming from clone-mates. Alternatively, when bacteria live in a group and the success of the individual is tied to the success of the local population—for example, because cells from more productive patches are more likely to colonize new patches—then the evolutionary interests of the individual and the group are aligned and groups containing cheats may die out.

Through the use of powerful techniques such as genomic sequencing, we are learning a huge amount about the mechanisms underlying bacterial social behaviors. But while we know a lot about how gene expression and protein translation are controlled, we still do not know the answers to basic questions about how these behaviors evolved, how they are maintained in natural populations, and whether they are social in nature. These questions matter, not least because in the case of pathogenic bacteria, they influence virulence and antimicrobial resistance.

**Testing Whether a Microbial Behavior Is Social**

To determine whether a microbial behavior is social, a number of key experimental steps need to be followed. First, defined mutants resulting in the abolition or reduction of the behavior need to be identified to demonstrate reduced fitness in an environment where the behavior provides a benefit to cells (Fig. 2). For example, in low iron conditions, siderophore mutants grow poorly compared to wild-type cells. Second, when mutants and wild-type cells are grown in mixed populations, mutant fitness should increase as they exploit wild-type cells that are producing useful molecules (Fig. 2). Finally, the fitness of a particular type of mutant should negatively correlate with its starting proportion in a mixed population. Put simply, as social cheats become more common, fewer wild-type producing cells will be present within the population to be exploited, resulting in decreased benefits to cheating.

West and his collaborators empirically demonstrated cooperative social behaviors in the form of siderophore production in *Pseudomonas aeruginosa*, and Joan Strassmann and David Queller at Washington University in St. Louis, Mo., revealed cooperative reproduction in slime
molds. When slime molds exhaust available nutrients, they produce a specialized fruiting body. Some cells differentiate into reproductive spores, while other cells form a stalk that holds the spore population aloft. The stalk increases the distance spores can disperse, maximizing their chances of finding a favorable habitat to germinate. Although stalk cells do not themselves reproduce, cooperative genotypes of slime mold differentiate into stalk and spore cells with equal probability. However, some cheating mutants preferentially become spores, increasing their fitness relative to cooperators.

The production of siderophores, small molecules that scavenge iron, by *P. aeruginosa* demonstrates cooperation in a more fundamental way. Where growth is restricted by iron availability, wild-type production of siderophores can be exploited by siderophore-null mutants, which act as social cheats. This neatly demonstrates that producing a costly public good can be classified as a social trait and implies that social behaviors may be common in bacteria.

**Quorum Sensing Provides Another Example of Social Behavior**

Our work focuses on bacterial quorum sensing (QS), a process whereby cells communicate with each other via diffusible signal molecules. These signals coordinate a wide range of behaviors at the population or group level. In a range of gram-negative and gram-positive bacterial species, QS signals regulate the production of extracellular “public goods,” including nutrient-scavenging molecules, toxins, immune suppressants, and surfactants that aid cellular motility.

Signal molecules are usually produced at a basal rate that does not induce these behaviors. However, when a population reaches a high density, the levels of the signal molecule also increase, leading to a positive feedback mechanism, which results in a considerable increase in signal and QS-controlled factors (Fig. 3). This coordinating function of QS and its link with public goods production indicate that it is a social behavior.

The fitness benefits of QS in *P. aeruginosa* are density dependent, and QS in both *P. aeruginosa* and *Staphylococcus aureus* is both costly and exploitable by cheats. Using a synthetic growth medium where QS is important for bacterial growth due to production of costly exoproteases, wild-type *P. aeruginosa* populations grow well, but populations of lasR mutants, which do not respond to QS signals, grow poorly. Crucially, in mixed culture, lasR mutants act as cheats: they have a fitness advantage because they exploit the exoprotease production of wild-type cells, ac-

---

**FIGURE 3**

The core dynamics of quorum sensing. (A) At low cell densities, a low level of signal is secreted and this is insufficient to activate a QS receptor protein. (B) As the number of bacteria in a space increases so does the level of signal. (C) At a given threshold (quorum) the signal can activate the receptor, which in turn causes a greater production of signal and receptor as well as up-regulating QS controlled genes, either directly or through secondary messengers.
AUTHOR PROFILE

Diggle: Plagues, Misdemeanors, and a Long-Term Interest in Bacteria

Steve Diggle traces his dual passion for microbiology and music to his primary school teacher Mrs. Liversage, who taught his class about the Great Plague of London of 1665 and, separately, showed him how to play a few chords on a guitar. “From that moment I developed an unhealthy obsession with plague and read lots of books on the subject. Learning about the plague when I was 10 years old was simply a revelation to me,” he says.

Diggle’s interest in bacteria continues, as does his guitar (bass) playing. At age 19, he and several of his friends formed a progressive rock band called Mr Meaner. “The name was supposed to be an amusing play on the word misdemeanor,” he says. “It was funny for about 10 minutes and then it became annoying, but we stuck with it.” After calling it a day in 1995, Mr Meaner recently reformed to record what they should have done 20 years ago. “Being in a band teaches you skills that...are important for science,” he says. “Perseverance...coping with rejection...collaborating on projects...being able to give and take criticism, keeping your feet on the ground, and not getting too bothered about things.”

Diggle is associate professor in the University of Nottingham Centre for Biomolecular Sciences, where he studies the social behavior of microbes—specifically, cooperation and communication, and their implications for microbial virulence. He also is interested in ways to treat infections and is collaborating with colleagues in the School of English to determine whether medieval and Anglo-Saxon recipes will work against organisms that are resistant to conventional antimicrobial agents.

Diggle, 44, was born and grew up in Stockport, an industrial town in northwest England, about seven miles from Manchester, which accounts for him being a Manchester United fan. His parents, now retired, were high school teachers, and his grandmother, who died this year at 100, was a particular inspiration to him. “She was just a remarkable lady who lived through a lot,” he says. “She was a missionary in Ethiopia in the late 1960s when it was a dangerous place to be. She and my granddad pretty much sold everything to go and help people, which I always found amazing.”

Diggle was not a high school standout. “I left with pretty much no qualifications,” he says. At 17, he began working in a small company that isolated a compound from rabbits that was used to test the clotting time of blood. “My job was to remove 200 brains from rabbit heads that we got every day from the local abattoir,” he says. “I got pretty fast at it.”

After 18 months, Diggle moved to work at Withington Hospital before moving to the Paterson Institute for Cancer Research, also in Manchester, he says. “However, I knew I needed to get a degree to move on.” Before attending Salford University full time in 1993, he did a “one day a week access course in science for two years,” he says. He graduated from Salford in 1997 at age 27. He earned his doctorate from the University of Nottingham in 2001, and then did postdoctoral research until 2006.

Diggle’s wife Fran teaches biology in an independent school, and their son, Angus, is 12. While the band still takes up most of his free time, he also likes to read. “I’m a bit of a Tolkien nerd, and I like the Game of Thrones novels and TV series,” he says.

Marlene Cimons
Marlene Cimons lives and writes in Bethesda, Md.

Diggle’s interest in bacteria continues, as does his guitar (bass) playing. At age 19, he and several of his friends formed a progressive rock band called Mr Meaner. “The name was supposed to be an amusing play on the word misdemeanor,” he says. “It was funny for about 10 minutes and then it became annoying, but we stuck with it.” After calling it a day in 1995, Mr Meaner recently reformed to record what they should have done 20 years ago. “Being in a band teaches you skills that...are important for science,” he says. “Perseverance...coping with rejection...collaborating on projects...being able to give and take criticism, keeping your feet on the ground, and not getting too bothered about things.”

Diggle is associate professor in the University of Nottingham Centre for Biomolecular Sciences, where he studies the social behavior of microbes—specifically, cooperation and communication, and their implications for microbial virulence. He also is interested in ways to treat infections and is collaborating with colleagues in the School of English to determine whether medieval and Anglo-Saxon recipes will work against organisms that are resistant to conventional antimicrobial agents.

Diggle, 44, was born and grew up in Stockport, an industrial town in northwest England, about seven miles from Manchester, which accounts for him being a Manchester United fan. His parents, now retired, were high school teachers, and his grandmother, who died this year at 100, was a particular inspiration to him. “She was just a remarkable lady who lived through a lot,” he says. “She was a missionary in Ethiopia in the late 1960s when it was a dangerous place to be. She and my granddad pretty much sold everything to go and help people, which I always found amazing.”

Diggle was not a high school standout. “I left with pretty much no qualifications,” he says. At 17, he began working in a small company that isolated a compound from rabbits that was used to test the clotting time of blood. “My job was to remove 200 brains from rabbit heads that we got every day from the local abattoir,” he says. “I got pretty fast at it.”

After 18 months, Diggle moved to work at Withington Hospital before moving to the Paterson Institute for Cancer Research, also in Manchester, he says. “However, I knew I needed to get a degree to move on.” Before attending Salford University full time in 1993, he did a “one day a week access course in science for two years,” he says. He graduated from Salford in 1997 at age 27. He earned his doctorate from the University of Nottingham in 2001, and then did postdoctoral research until 2006.

Diggle’s wife Fran teaches biology in an independent school, and their son, Angus, is 12. While the band still takes up most of his free time, he also likes to read. “I’m a bit of a Tolkien nerd, and I like the Game of Thrones novels and TV series,” he says.

Marlene Cimons
Marlene Cimons lives and writes in Bethesda, Md.

According to our findings and those of Martin Schuster at Oregon State University in Corvallis and his collaborators.

Similarly, when mice with burned skin or with chronic wounds are infected with P. aeruginosa, we found that lasR mutants act as cheats and can invade this bacterial population within days. In waxmoth larvae (waxworm) infections, we showed a similar pattern of sociality for S. aureus agr mutants. The mutants are less fit than their wild-type counterparts in monoculture infections but demonstrate social cheating when in mixed infection.

These results suggest that, during infection, QS cheats can exploit public goods and resources produced by a cooperating population to gain a fitness benefit. In addition, mixed infections of cooperating and cheating cells of P. aeruginosa and S. aureus are less virulent than are unmixed infections. Therefore, the spread of cheats in a population can significantly alter the outcome of an infection. Even within a single species, significant phenotypic diversity can evolve during infection, magnifying the importance of this phenomenon. Social evolution theory can help to explain how and why such diversity arises and its implications for virulence, infection, and antibiotic resistance.

Such results also help to explain why QS mutants arise in clinical infections even though such loss-of-function mutations might appear to be detrimental to fitness. Both agr and lasR mutants
have been isolated from different types of infections, particularly chronic infections. The predominant types of mutation affect the ability of cells to respond to signals, rather than to make signal molecules. Theory suggests such “signal-blind” mutants make better cheats because they can no longer respond to signal and thus cannot cooperate with wild-type bacteria to make costly public goods.

However, although QS systems can be social both in vitro and in vivo, it is important to realize that the growth environment can alter the social nature of traits. For example, we recently showed that, in porcine lung tissues, QS may not provide any social benefits. In this system, lasR mutants do not cheat, they simply appear to grow better.

This result could mean that QS P. aeruginosa mutants in infected cystic fibrosis patients arise due to adaptation rather than through social cheating. Furthermore, QS in P. aeruginosa does more than control traits that can be considered public goods. Some enzymes controlled by QS are intracellular, and break down nutrients for growth within the cell, providing direct benefits for the producer cell. By regulating private goods, this metabolic component of the QS system may well restrain social cheating, according to Peter Greenberg of the University of Washington in Seattle and his collaborators. Therefore the growth environment can change the social dynamics of traits, highlighting the need for further testing which traits are social in different environments.

Eric J. G. Pollitt is a Research Fellow at the University of Sheffield, Freya Harrison is a Research Fellow, and Stephen P. Diggle is an Associate Professor at the School of Life Sciences, University of Nottingham, Nottingham, United Kingdom.

Suggested Reading


Methane Fuels Deep-Sea, Rock-Hosted Ecosystem

Symbiotic clumps of methanotrophic archaea and sulfate-reducing bacteria anaerobically oxidize about 80% of the methane released from seafloor seeps

Jeffrey J. Marlow

Far beneath the ocean surface, vast quantities of methane percolate through the Earth’s crust, following fractures and fissures into the shallow subsurface and frequently into the depths of the sea. These features are marine methane seeps, important components of the planet’s climate regulation system. Methane moving through these seeps derives from the breakdown of larger organic molecules nestled within the crust and from closer-range biological production.

What concerns biogeochemists is the fate of methane when it arrives at the seafloor. Unchecked, it can enter the water column and, after rising through the gauntlet of aerobic methanotrophic organisms, enter the atmosphere. More influential than carbon dioxide on a per-molecule basis, methane is the third most significant contributor to greenhouse warming, its atmospheric concentration rising in part because of proliferating anthropogenic sources.

However, much of the methane rising through the seafloor is consumed before it ever reaches the water. Microbial consortia consisting mainly of anaerobic methanotrophic archaea (ANME) and sulfate-reducing bacteria (SRB) consume an estimated 80% of sub-seafloor methane in a process known as the anaerobic oxidation of methane (AOM). Despite their fundamental role in carbon and climate dynamics, the microorganisms responsible for AOM were discovered relatively recently, within the last two decades. Hence, much remains to be learned about the underlying ANME-SRB partnership. For instance, which components of the carbon and sulfur metabolisms are conducted by each partner? How is energy shared between the archaeal and bacterial constituents?

Classic View of Methane-Sulfur Cycle Vague on Details

The “classic” view of AOM, summarized here in equation 1, is a bulk relationship, as the precise contributions of each microbial member are still unknown.

\[
\text{CH}_4(aq) + \text{SO}_4^{2-}(aq) \rightarrow \text{HCO}_3^-(aq) + \text{HS}^-(aq) + \text{H}_2\text{O}(l) \quad \text{Eq. 1}
\]

One result of this reaction is that local alkalinity rises. Another is that increased levels of bicarbonate ions can react with calcium cations in seawater to generate solid calcium carbonate (Eq. 2), leading, over time, to a landscape of carbonate precipitates:

\[
2\text{HCO}_3^-(aq) + \text{Ca}^{2+}(aq) \leftrightarrow \text{CaCO}_3(s) + \text{CO}_2(aq) + \text{H}_2\text{O}(l) \quad \text{Eq. 2}
\]

Friable, centimeter-scale nodules populate shallow sediment horizons surrounding active seeps. Consolidated carbonate pavements can cover

SUMMARY

➤ Microbial consortia at and below the seafloor thrive on methane emerging from underground seeps, oxidizing much of this gas long before it reaches the atmosphere.
➤ Newly discovered communities actively consume methane while living inside carbonate rocks.
➤ Endolithic methanotrophic microbes recovered from Hydrate Ridge off the Oregon Coast serve as an energy source for other constituents of this marine ecosystem.
➤ Marine Benthic Group B archaea are the most prominent lineages in off-seep background sediments.
➤ Among bacteria, Deltaproteobacteria are the most abundant lineage in nearly all samples from active seep sites, reflecting their role as sulfate-reducing partners in anaerobic methane oxidation.
much of the seafloor and may ultimately form large mounds that rise hundreds of meters above the ocean bottom. At other sites where upward fluid flow has ceased, similar carbonate structures may lie just beneath the seafloor.

Earlier analyses of AOM in the marine environment focused on sediments that block the path of escaping methane, shaping our knowledge of AOM dynamics, rates, energetics, and constituent organisms. However, when viewed from a structural geological perspective, these sediments may account for only a small fraction of the methane-infused volume, raising other questions. For instance, could methane seep carbonates be more than a passive repository for biologically active sediments? Might metabolically active microbes inhabit these structures, and could they too be involved in the global methane cycle?

**Intact Microbes Occupy Carbonate Rocks**

Endoliths, organisms that inhabit the pores and fissures within rocks, are found in a diverse array of sites, ranging from Antarctic sandstones to deep-ocean basalts. Such habitats may protect their inhabitants from radiation or predators at the surfaces of those rocks, and they might also furnish their inhabitants with redox-active, energy-yielding metabolites. While they frequently face physicochemical and energetic challenges, endoliths are increasingly viewed as a pervasive form of life on this planet.

In search of carbonate rock-based AOM, we collected samples from the methane seeps of Hydrate Ridge off the Oregon coast during two research cruises aboard the R/V Atlantis. This formation consists of an oblong mound of carbonate rock protruding about 100 m above the continental slope, topping out at a depth of 600 m (Fig. 1). Just beneath the seafloor lie extensive deposits of structurally stable, solid methane hydrate, giving the site its name.

Geologists, oceanographers, and biologists began studying Hydrate Ridge decades ago. Its scale, dynamism, and bizarre menagerie make the site a continuing source of scientific discovery. Our dual expeditions, led by Lisa Levin and Greg Rouse of the Scripps Institution of Oceanography, Victoria Orphan of the California Institute of Technology, and Anthony Rathburn of Indiana State University, were part of an effort to determine how various constituents of the methane seep ecosystem, including methanotrophic consortia, eukaryotic microbes, and macrofauna, interact.

Using the manned submersible Alvin and the robotic craft Jason, we recovered sediment, nodules, and carbonate rocks from actively venting locations, sediment and carbonate rocks from low-activity sites, and sediment from a background seafloor location that bore no signs of past or present methane seep activity. By analyzing those samples, our team sought to demonstrate how methanotrophic microbes form the base of the trophic pyramid at Hydrate Ridge, serving as prey or generating metabolic by-products that can provide energy for other constituents.

Despite their foundational role, the habitat range of these primary producers is poorly constrained. When we looked for microbial biomass in rock fractions isolated from the surface environment, we were surprised by the abundance of microbes. At active seep sites, we observed larger aggregates of ANME-SRB consortia within carbonate rocks than in sediment (10.2 and 6.51 μm average diameters, respectively; Fig. 2). These aggregates are also more abundant in carbonate rocks (with approximately 500 aggregates per mg) than in the corresponding sediment (which exhibited 320 per mg). Yet when we developed three-dimensional reconstructions of aggregates, we found a more diffuse arrangement in endolithic consortia, with a packing density roughly 25% that of active sediment aggregates.

Taken together, endolithic aggregates from active seep sites, which are larger and more abundant, but less densely packed, account for about 40% more biomass than those in active sediments. Fluorescent in situ hybridization (FISH) experiments confirm that these clumps of cells contain the same ANME and SRB lineages as do “traditional” sediment-based AOM consortia.

**Evidence for Active Endolithic Microbial Metabolism**

Although these observations pointed to intact cells in pore spaces and cavities of rocks, the question remained whether these endolithic inhabitants are metabolically active. We approached this question from two directions, looking at both catabolism (reactions that yield energy) and anabolism (those that are biosynthetic).

We injected radiolabeled $^{14}$CH$_4$ into small bottles containing sediment or carbonate rock from
both active and seemingly inactive seep sites as well as killed samples (which would detect any abiotic processes). After several days, remarkably, all samples (except for the controls) exhibited measureable rates of AOM. The sediment community oxidized methane at a rate of 193.6 nmol/cm$^3$ day, the carbonate community at 80.9 nmol/cm$^3$ day, and sediment and carbonate collected outside the area of active methane emission only 7.5 and 11.8 nmol/cm$^3$ day, respectively. Sediment samples from other active methane seep sites around the world typically exhibit oxidation rates of a similar order of magnitude. Not only were supposedly “inactive” sites not quite as dormant as we expected, but the overall oxidation rates of endolithic communities proved comparable to what can be measured in sediments.

To study whether the endolithic biomass actively incorporates new material, we injected $^{15}$N-labeled ammonium ions into carbonate rocks recovered from active seep, opting for labeled ammonium instead of carbon because only about 1% of methane carbon transfers into the biomass. Thus, labeled nitrogen is more practical for measuring anabolic activities in such settings, particularly when faced with microorganisms whose doubling times are typically measured in months. We waited 27 months after injecting the labeled ammonium to examine these communities, using FISH and a nano-secondary ion mass spectrometer (nanoSIMS), to determine how much $^{15}$N was taken up and where it went. The $^{15}$N abundances were as high as 88 atomic percent, revealing methanotrophic organisms that doubled multiple times during those 27 months. This catabolic and anabolic activity indicated that endolithic methanotrophs are indeed active around deep-sea methane seeps.
Archaea Mobilize Methane, while Bacteria Are More Metabolically Versatile

Although all of our sample types—sediments and carbonate rocks from actively venting and “low-activity” sites—metabolized methane, the cell abundances and methane consumption rates ranged widely. These differences prompted us to analyze how the two primary variables—the degree of seep activity and the type of physical substrate—shape microbial compositions at different sites.

To learn which microbes inhabit each setting, we analyzed full-length 16S rRNA gene sequences from each of 12 different locations (Fig. 3). By analyzing duplicate samples from sites separated by several kilometers, we found a surprising degree of similarity, suggesting that the chemical and physical variables we were evaluating seemed to matter more in shaping the community structure than localized heterogeneity.

Some lineages of methane-oxidizing communities are more common in particular environments than in others. While ANME groups dominate the archaeal sequences from all active sites, Marine Benthic Group B archaea are the most prominent lineage that we recovered from off-seep background sediments. Like other researchers, we found that the ANME-1 are more abundant in areas that receive lower methane input, whereas ANME-2 and -3 are found closer to sites that are actively producing methane. A relatively high abundance of Marine Group I organisms, which are pervasive in the deep-water column, is found in carbonate rocks at low seepage sites.
Map of sampling locations at and around Hydrate Ridge, accompanied by images showing the general locations of selected samples. (AS and AC indicate sediment and carbonate from actively seeping sites, respectively; LS and LC stand for sediment and carbonate from low seepage activity locations; OS refers to off-seep sediment. Nodules, indicated by the N postscript, were collected from the same sample core as the active sediment.) Samples LC-5189, LC-5164, AC-5120, AS-5119, and AS-5119N were collected from Hydrate Ridge north (mound summit 600 m depth); samples AC-3439, LS-3433, LC-3662, AS-3730, and AS-3730N were collected from Hydrate Ridge south (mound top ~780 m depth); samples OS-3582 and OS-3487 were collected off-seep from a water depth of ~600 m. Hydrate Ridge north and south sampling sites were located approximately 12 km apart. Base map is derived from Global Multi-Resolution Topography (GMRT, Ryan et al., 2009; GeoMapApp); contour lines represent 100 m of depth, and each minute of latitude represents 1.85 km. In the images, push cores are 10 cm in diameter for scale. (Reprinted with permission from J. J. Marlow et al., Frontiers Aquatic Microbiol. 1:44, doi:10.3389/tmars.2014;00044.)
implying a “seeding” of microbial constituents in substrates exposed at the seafloor.

Among bacteria, Deltaproteobacteria are the most abundant class in nearly all samples from active sites, reflecting the role of members within this lineage as sulfate-reducing partners in AOM. At active seep sediments, sulfur-oxidizing *Sulfurovum* spp. are prevalent, suggesting an interactive sulfur cycle with sulfate-reducing AOM at deeper sediment horizons. Such organisms are nearly absent from other samples—most notably, active seep carbonate rocks—suggesting there could be a physical substrate-based control on *Sulfurovum*.

Among the 12 sampled microbial communities, seep activity level correlates with the distribution of archaea, while bacterial representatives appear to be more influenced by the physical nature of the habitat. This finding fits well with our model of marine methane-based community dynamics while also broadening our understanding of how methane seep ecosystems work. Archaeal ANME species mobilize methane and act as primary producers, suggesting reliance upon methane flux. Bacteria appear to be more metabolically versatile, with some combination of mineralogy, porosity, and permeability influencing community structure more than methane supply or links to particular archael partners.

### Endolithic Habitats

The abundance of these endolithic consortia make them an attractive food source for organisms higher up the trophic pyramid, according to expedition participant Andrew Thurber, now at Oregon State University in Corvallis, who analyzed stable carbon isotope distribution patterns. Researchers tracing the flow of carbon through ecosystems find that the carbon profile of methane is isotopically “light,” meaning its ratio of $^{12}\text{C}$ to $^{13}\text{C}$ is lower than that of most carbon in the biosphere. This trait makes it relatively easy to track. When examining the carbon isotopic profile of polychaete worms of the *Dorvilleidae* family, which populate sediments and rocks around active methane seeps, other scientists typically found extremely low bulk $^{12}\text{C}/^{13}\text{C}$ ratios, indicating that the carbon initially came from methane.

However, this result was not specific enough to directly link AOM aggregates to the Dorvilleids, since the worms could have been acquiring isotopically light carbon from other primary produc-

ers, such as aerobic methanotrophs near the surface of the seafloor or other autotrophs that incorporated carbon that had been previously processed by ANME. Thus, Thurber and his collaborators examined fatty acids from the worms, searching for biomolecules that came from sulfate-reducing bacteria associated with ANMEs.

One of those molecules, an 18-carbon fatty acid, provided the missing link. Although worms cannot make this 18-carbon molecule, they can make its 16-carbon precursor. By measuring the isotopic composition of these two fatty acids, Thurber and his collaborators showed that the only plausible source of the carbon within the 18-carbon molecule was archaeal biomass. The result indicates the macrofaunal worms dine on AOM aggregates within methane seep sediments and porous carbonate rocks, mobilizing carbon within this cold, dark ecosystem.

### Outlook

Methane-derived carbonate mounds like those found at Hydrate Ridge are pervasive along continental margins. Indeed, the recent discovery of active seeps in the Atlantic Ocean indicates that exploration of these environments is still in its infancy. Finding active methanotrophic microorganisms in pore spaces within these large mounds, which can reach hundreds of meters in height and diameter, has enlarged our understanding of deep-sea methane fluxes.

Jeffrey J. Marlow is a Ph.D. candidate in the Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena.

### Suggested Reading


Methane Fuels Deep-Sea, Rock-Hosted Ecosystem

Symbiotic clumps of methanotrophic archaea and sulfate-reducing bacteria anaerobically oxidize about 80% of the methane released from seafloor seeps

Jeffrey J. Marlow

Far beneath the ocean surface, vast quantities of methane percolate through the Earth’s crust, following fractures and fissures into the shallow subsurface and frequently into the depths of the sea. These features are marine methane seeps, important components of the planet’s climate regulation system. Methane moving through these seeps derives from the breakdown of larger organic molecules nestled within the crust and from closer-range biological production.

What concerns biogeochemists is the fate of methane when it arrives at the seafloor. Unchecked, it can enter the water column and, after rising through the gauntlet of aerobic methanotrophic organisms, enter the atmosphere. More influential than carbon dioxide on a per-molecule basis, methane is the third most significant contributor to greenhouse warming, its atmospheric concentration rising in part because of proliferating anthropogenic sources.

However, much of the methane rising through the seafloor is consumed before it ever reaches the water. Microbial consortia consisting mainly of anaerobic methanotrophic archaea (ANME) and sulfate-reducing bacteria (SRB) consume an estimated 80% of sub-seafloor methane in a process known as the anaerobic oxidation of methane (AOM). Despite their fundamental role in carbon and climate dynamics, the microorganisms responsible for AOM were discovered relatively recently, within the last two decades. Hence, much remains to be learned about the underlying ANME-SRB partnership. For instance, which components of the carbon and sulfur metabolisms are conducted by each partner? How is energy shared between the archaean and bacterial constituents?

Classic View of Methane-Sulfur Cycle Vague on Details

The “classic” view of AOM, summarized here in equation 1, is a bulk relationship, as the precise contributions of each microbial member are still unknown.

\[
\text{CH}_4(aq) + \text{SO}_4^{2-}(aq) \rightarrow \text{HCO}_3^-(aq) + \text{HS}^-(aq) + \text{H}_2\text{O}(l) \quad \text{Eq. 1}
\]

One result of this reaction is that local alkalinity rises. Another is that increased levels of bicarbonate ions can react with calcium cations in seawater to generate solid calcium carbonate (Eq. 2), leading, over time, to a landscape of carbonate precipitates:

\[
2\text{HCO}_3^-(aq) + \text{Ca}^{2+}(aq) \leftrightarrow \text{CaCO}_3(s) + \text{CO}_2(aq) + \text{H}_2\text{O}(l) \quad \text{Eq. 2}
\]

Friable, centimeter-scale nodules populate shallow sediment horizons surrounding active seeps. Consolidated carbonate pavements can cover

SUMMARY

➤ Microbial consortia at and below the seafloor thrive on methane emerging from underground seeps, oxidizing much of this gas long before it reaches the atmosphere.
➤ Newly discovered communities actively consume methane while living inside carbonate rocks.
➤ Endolithic methanotrophic microbes recovered from Hydrate Ridge off the Oregon Coast serve as an energy source for other constituents of this marine ecosystem.
➤ Marine Benthic Group B archaea are the most prominent lineages in off-seep background sediments.
➤ Among bacteria, Deltaproteobacteria are the most abundant lineage in nearly all samples from active seep sites, reflecting their role as sulfate-reducing partners in anaerobic methane oxidation.
much of the seafloor and may ultimately form large mounds that rise hundreds of meters above the ocean bottom. At other sites where upward fluid flow has ceased, similar carbonate structures may lie just beneath the seafloor.

Earlier analyses of AOM in the marine environment focused on sediments that block the path of escaping methane, shaping our knowledge of AOM dynamics, rates, energetics, and constituent organisms. However, when viewed from a structural geological perspective, these sediments may account for only a small fraction of the methane-infused volume, raising other questions. For instance, could methane seep carbonates be more than a passive repository for biologically active sediments? Might metabolically active microbes inhabit these structures, and could they too be involved in the global methane cycle?

**Intact Microbes Occupy Carbonate Rocks**

Endoliths, organisms that inhabit the pores and fissures within rocks, are found in a diverse array of sites, ranging from Antarctic sandstones to deep-ocean basalts. Such habitats may protect their inhabitants from radiation or predators at the surfaces of those rocks, and they might also furnish their inhabitants with redox-active, energy-yielding metabolites. While they frequently face physicochemical and energetic challenges, endoliths are increasingly viewed as a pervasive form of life on this planet.

In search of carbonate rock-based AOM, we collected samples from the methane seeps of Hydrate Ridge off the Oregon coast during two research cruises aboard the R/V Atlantis. This formation consists of an oblong mound of carbonate rock protruding about 100 m above the continental slope, topping out at a depth of 600 m (Fig. 1). Just beneath the seafloor lie extensive deposits of structurally stable, solid methane hydrate, giving the site its name.

Geologists, oceanographers, and biologists began studying Hydrate Ridge decades ago. Its scale, dynamism, and bizarre menagerie make the site a continuing source of scientific discovery. Our dual expeditions, led by Lisa Levin and Greg Rouse of the Scripps Institution of Oceanography, Victoria Orphan of the California Institute of Technology, and Anthony Rathburn of Indiana State University, were part of an effort to determine how various constituents of the methane seep ecosystem, including methanotrophic consortia, eukaryotic microbes, and macrofauna, interact.

Using the manned submersible Alvin and the robotic craft Jason, we recovered sediment, nodules, and carbonate rocks from actively venting locations, sediment and carbonate rocks from low-activity sites, and sediment from a background seafloor location that bore no signs of past or present methane seep activity. By analyzing those samples, our team sought to demonstrate how methanotrophic microbes form the base of the trophic pyramid at Hydrate Ridge, serving as prey or generating metabolic by-products that can provide energy for other constituents.

Despite their foundational role, the habitat range of these primary producers is poorly constrained. When we looked for microbial biomass in rock fractions isolated from the surface environment, we were surprised by the abundance of microbes. At active seep sites, we observed larger aggregates of ANME-SRB consortia within carbonate rocks than in sediment (10.2 and 6.51 μm average diameters, respectively; Fig. 2). These aggregates are also more abundant in carbonate rocks (with approximately 500 aggregates per mg) than in the corresponding sediment (which exhibited 320 per mg). Yet when we developed three-dimensional reconstructions of aggregates, we found a more diffuse arrangement in endolithic consortia, with a packing density roughly 25% that of active sediment aggregates.

Taken together, endolithic aggregates from active seep sites, which are larger and more abundant, but less densely packed, account for about 40% more biomass than those in active sediments. Fluorescent in situ hybridization (FISH) experiments confirm that these clumps of cells contain the same ANME and SRB lineages as do “traditional” sediment-based AOM consortia.

**Evidence for Active Endolithic Microbial Metabolism**

Although these observations pointed to intact cells in pore spaces and cavities of rocks, the question remained whether these endolithic inhabitants are metabolically active. We approached this question from two directions, looking at both catabolism (reactions that yield energy) and anabolism (those that are biosynthetic).

We injected radiolabelled $^{14}$CH$_4$ into small bottles containing sediment or carbonate rock from
both active and seemingly inactive seep sites as well as killed samples (which would detect any abiotic processes). After several days, remarkably, all samples (except for the controls) exhibited measurable rates of AOM. The sediment community oxidized methane at a rate of 193.6 nmol/cm$^3$ day, the carbonate community at 80.9 nmol/cm$^3$ day, and sediment and carbonate collected outside the area of active methane emission only 7.5 and 11.8 nmol/cm$^3$ day, respectively. Sediment samples from other active methane seep sites around the world typically exhibit oxidation rates of a similar order of magnitude. Not only were supposedly “inactive” sites not quite as dormant as we expected, but the overall oxidation rates of endolithic communities proved comparable to what can be measured in sediments.

To study whether the endolithic biomass actively incorporates new material, we injected $^{15}$N-labeled ammonium ions into carbonate rocks recovered from active seep, opting for labeled ammonium instead of carbon because only about 1% of methane carbon transfers into the biomass. Thus, labeled nitrogen is more practical for measuring anabolic activities in such settings, particularly when faced with microorganisms whose doubling times are typically measured in months. We waited 27 months after injecting the labeled ammonium to examine these communities, using FISH and a nano-secondary ion mass spectrometer (nanoSIMS), to determine how much $^{15}$N was taken up and where it went. The $^{15}$N abundances were as high as 88 atomic percent, revealing methanotrophic organisms that doubled multiple times during those 27 months. This catabolic and anabolic activity indicated that endolithic methanotrophs are indeed active around deep-sea methane seeps.
Archaea Mobilize Methane, while Bacteria Are More Metabolically Versatile

Although all of our sample types—sediments and carbonate rocks from actively venting and “low-activity” sites—metabolized methane, the cell abundances and methane consumption rates ranged widely. These differences prompted us to analyze how the two primary variables—the degree of seep activity and the type of physical substrate—shape microbial compositions at different sites.

To learn which microbes inhabit each setting, we analyzed full-length 16S rRNA gene sequences from each of 12 different locations (Fig. 3). By analyzing duplicate samples from sites separated by several kilometers, we found a surprising degree of similarity, suggesting that the chemical and physical variables we were evaluating seemed to matter more in shaping the community structure than localized heterogeneity.

Some lineages of methane-oxidizing communities are more common in particular environments than in others. While ANME groups dominate the archaeal sequences from all active sites, Marine Benthic Group B archaea are the most prominent lineage that we recovered from off-seep background sediments. Like other researchers, we found that the ANME-1 are more abundant in areas that receive lower methane input, whereas ANME-2 and -3 are found closer to sites that are actively producing methane. A relatively high abundance of Marine Group I organisms, which are pervasive in the deep-water column, is found in carbonate rocks at low seepage sites.
FIGURE 3

Map of sampling locations at and around Hydrate Ridge, accompanied by images showing the general locations of selected samples. (AS and AC indicate sediment and carbonate from actively seeping sites, respectively; LS and LC stand for sediment and carbonate from low seepage activity locations; OS refers to off-seep sediment. Nodules, indicated by the N postscript, were collected from the same sample core as the active sediment.) Samples LC-5189, LC-5164, AC-5120, AS-5119, and AS-5119N were collected from Hydrate Ridge north (mound summit 600 m depth); samples AC-3439, LS-3433, LC-3662, AS-3730, and AS-3730N were collected from Hydrate Ridge south (mound top ~780 m depth); samples OS-3582 and OS-3487 were collected off-seep from a water depth of ~600 m. Hydrate Ridge north and south sampling sites were located approximately 12 km apart. Base map is derived from Global Multi-Resolution Topography (GMRT, Ryan et al., 2009; GeoMapApp); contour lines represent 100 m of depth, and each minute of latitude represents 1.85 km. In the images, push cores are 10 cm in diameter for scale. (Reprinted with permission from J. J. Marlow et al., Frontiers Aquatic Microbiol. 1:44, doi:10.3389/tmars.2014;00044.)
implying a “seeding” of microbial constituents in substrates exposed at the seafloor.

Among bacteria, Deltaproteobacteria are the most abundant class in nearly all samples from active sites, reflecting the role of members within this lineage as sulfate-reducing partners in AOM. At active seep sediments, sulfur-oxidizing *Sulfurovum* spp. are prevalent, suggesting an interactive sulfur cycle with sulfate-reducing AOM at deeper sediment horizons. Such organisms are nearly absent from other samples—most notably, active seep carbonate rocks—suggesting there could be a physical substrate-based control on *Sulfurovum*.

Among the 12 sampled microbial communities, seep activity level correlates with the distribution of archaea, while bacterial representatives appear to be more influenced by the physical nature of the habitat. This finding fits well with our model of marine methane-based community dynamics while also broadening our understanding of how methane seep ecosystems work. Archaeal ANME species mobilize methane and act as primary producers, suggesting reliance upon methane flux. Bacteria appear to be more metabolically versatile, with some combination of mineralogy, porosity, and permeability influencing community structure more than methane supply or links to particular archaeal partners.

**Endolithic Habitats**

The abundance of these endolithic consortia make them an attractive food source for organisms higher up the trophic pyramid, according to expedition participant Andrew Thurber, now at Oregon State University in Corvallis, who analyzed stable carbon isotope distribution patterns. Researchers tracing the flow of carbon through ecosystems find that the carbon profile of methane is isotopically “light,” meaning its ratio of $^{12}\text{C}$ to $^{13}\text{C}$ is lower than that of most carbon in the biosphere. This trait makes it relatively easy to track. When examining the carbon isotopic profile of polychaete worms of the *Dorvilleidae* family, which populate sediments and rocks around active methane seeps, other scientists typically found extremely low bulk $^{12}\text{C}/^{13}\text{C}$ ratios, indicating that the carbon initially came from methane.

However, this result was not specific enough to directly link AOM aggregates to the Dorvilleids, since the worms could have been acquiring isotopically light carbon from other primary producers, such as aerobic methanotrophs near the surface of the seafloor or other autotrophs that incorporated carbon that had been previously processed by ANME. Thus, Thurber and his collaborators examined fatty acids from the worms, searching for biomolecules that came from sulfate-reducing bacteria associated with ANMEs. One of those molecules, an 18-carbon fatty acid, provided the missing link. Although worms cannot make this 18-carbon molecule, they can make its 16-carbon precursor. By measuring the isotopic composition of these two fatty acids, Thurber and his collaborators showed that the only plausible source of the carbon within the 18-carbon molecule was archaeal biomass. The result indicates the macrofaunal worms dine on AOM aggregates within methane seep sediments and porous carbonate rocks, mobilizing carbon within this cold, dark ecosystem.

**Outlook**

Methane-derived carbonate mounds like those found at Hydrate Ridge are pervasive along continental margins. Indeed, the recent discovery of active seeps in the Atlantic Ocean indicates that exploration of these environments is still in its infancy. Finding active methanotrophic microorganisms in pore spaces within these large mounds, which can reach hundreds of meters in height and diameter, has enlarged our understanding of deep-sea methane fluxes.

*Jeffrey J. Marlow is a Ph.D. candidate in the Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena.*

**Suggested Reading**


Are you considering a career outside of academia?

Check out the ASM Headquarter's Fellowship Programs

ASM's Headquarter's Fellowship program provides an intense and multi-faceted training experience for individuals interested in exploring careers outside the traditional academic environment.

There are two opportunities being offered:

- Career Advancement Fellowship Program
  (Please note: Applicants must have graduated by 12/31/14)

- Professional Practice Industrial Fellowship Program

Applicants for the fellowship should have a broad interest in the field of microbiology and a willingness to learn about careers and professional development needs for individuals working in the microbial sciences field.

You can find more information and the online applications for the two opportunities at http://bit.ly/HQopp.
ASM Communications Committee Strategic Plan

The ASM Communications Committee has a new strategic plan, which will guide their activities for the next five years. The Committee oversees public outreach and media relations for the Society. Major projects include the Microbe World.org website, the USA Science and Engineering Festival, and working with members to produce and distribute their blogs and podcasts. The Council Policy Committee approved the plan during their fall meeting. The main goals outlined in the plan are:

Science and Society. ASM Communications will identify strategic, global issues and will provide timely information that informs and engages audiences.

Membership Empowerment. ASM Communications will empower ASM members to communicate confidently and effectively about science.

Leadership in Science Communication. ASM Communications will be a recognized leader in using evidence-based, innovative, and compelling strategies to exchange knowledge.

Capacity and Capabilities. ASM Communications will have the resources necessary to foster and sustain excellence.

Reputation. ASM Communications will ensure that ASM is known universally as a leader in the dissemination of and dialogue about reliable and relevant information about microbiology and related sciences.

Committee Chair Vincent Racaniello of Columbia University says that “the plan is essential for focusing our work in order to help us connect people with our science to increase their appreciation, understanding, and excitement about the world of microbes.” The committee’s initial efforts will focus on identifying topics on which the society should communicate, exploring improvements to the ASM website, and developing science communication training programs for members.

There are several opportunities to be involved with the Committee’s work. The committee is seeking members to advise on digital media and member empowerment projects. If you are interested in participating, please e-mail a statement of interest and your CV to communications@asmusa.org by 15 February.

ASM Speakers’ Bureau

ASM initiated a Speakers’ Bureau in late 2013 to educate ASM Student Chapters about microbiology-related career opportunities outside of academic research. Many students are interested to know what options are available to them after college, and it is the Speakers’ Bureau’s goal to inform them of the myriad of opportunities that they may not have considered. Over 90 ASM members from many different areas of microbiology have volunteered to speak to ASM Student Chapters about their particular careers. Speakers discuss why they chose their career path, career highlights, day-to-day responsibilities, and more.

This program is currently under the direction of Susan Bagley, Professor Emerita, Michigan Technological University. As committee chair, Bagley has been working hard to ensure that the Speakers’ Bureau appeals to students and fulfills their needs. She was excited to participate in this program to let students know about the career possibilities available to them. A key feature is readily available Speakers, whether in person or by video, so Student Chapters can benefit from this resource regardless of their proximity to speakers. Bagley envisions expansion of this program to include speakers and students outside of the United States and its use as an online career resource.

Since its launch, seven events have been held nationwide and three events are currently scheduled for late 2014. Based on the students’ and speakers’ feedback from these events, the Speakers’ Bureau has been deemed a success! The program is giving students invaluable insight into
careers so that they can make an informed decision upon graduation. Here is what attendees of past events had to say:

“[The speaker] did an excellent job in presenting her experience to both undergraduates and graduate students. I really feel she opened the door to industry careers, which are often forgotten about,” said a student from University of North Texas. Rosana Schafer, Associate Professor, West Virginia University, says “The students were appreciative of the opportunity to learn about the career options available to microbiologists in the field of biosafety and biosecurity, which many of them were completely unaware of prior to Dr. delaRosa’s visit.”

Experts are currently available to discuss their dynamic careers in areas of microbiology and immunology such as Biological Safety, Clinical Microbiology or Immunology, Food and Dairy Microbiology, Pharmaceuticals and Medical Devices, Public Health, Public Policy, and Quality Assurance/Quality Control.

The Speakers’ Bureau is currently seeking volunteers who work in bioremediation, biotechnology, indoor air quality, industrial microbiology, policy, technical sales, water, as well as the others listed above.

Learn more and Get Involved! ASM Members, if you would like to meet with a local ASM Student Chapter or meet via video call with remote Student Chapters, join the Speakers’ Bureau—see bit.ly/1wbNc9u.

ASM Student Chapters. To schedule a live event with a local speaker or a video call with a speaker of interest, please view the speakers’ directory profiles (bit.ly/ASMspeakers). Once you decide which speaker(s) you are interested in, please e-mail Nicole Jackson at professionalpractice@asmusa.org. ASM will contact the speaker to confirm availability. This event is of no cost to the Chapter. Please feel free to reach out to Ms. Jackson with any questions you may have.

Cases in Medical Microbiology and Infectious Diseases Fourth Edition

Authors: Peter H. Gilligan, Daniel S. Shapiro, and Melissa B. Miller

Cases in Medical Microbiology and Infectious Diseases is a proven resource for preparing for Part I of the National Board of Medical Examiners Exam and an excellent reference for infectious disease rotations. The cases are presented as “unknowns” and represent actual case presentations of patients the authors have encountered. Each case is accompanied by several questions to test knowledge in broad areas.

“A uniquely practical text that presents the diagnostic, pathogenic, prevention, therapeutic and public health aspects of clinical microbiology in the context of case based presentations. An essential resource for training the clinical microbiologist, pathology resident and infectious disease fellow.”
- David W. Craft PhD, Medical Director of Microbiology, Penn State Milton S. Hershey Medical Center

Instructors – please email books@asmusa.org to learn more about examining or adopting this title!

When you buy books directly from ASM Press, you support the society that supports the science of microbiology.

WEB: www.ASMscience.org | PHONE: 800-546-2416 | E-MAIL: books@asmusa.org

©2014 American Society for Microbiology Press
ASM Public Affairs

ASM Sends Letter to Congress on Omnibus Funding

On 12 November, ASM sent a letter to Congress supporting the passage of an FY 2015 Omnibus Appropriations bill before the end of the calendar year, rather than passing a continuing resolution based on FY 2014 levels that inadequately support innovation in science and technology. ASM requested the highest possible FY 2015 funding levels for the multiple federal agencies that provide support for science, including basic and applied microbiology: the National Institutes of Health (NIH), the National Science Foundation (NSF), the Department of Energy (DOE) Office of Science, Department of Agriculture (USDA) research programs, and Department of Defense (DOD) infectious disease programs. ASM said FY 2015 funding for the Centers for Disease Control and Prevention (CDC) and the Food and Drug Administration (FDA) must also be increased to protect the nation’s health and security. An FY 2015 Omnibus Appropriations bill that combines all agency appropriations into one bill is preferable to a partial, or full-year, continuing resolution, because it allows for increased funding and program continuity. The ASM letter is available on the ASM’s Public Policy website at http://www.asm.org/index.php/public-policy/2/statements-testimony/137-policy/93264-fy2015-omnibus.

ASM Statements on Ebola Virus Outbreak

On 29 October, the ASM Public and Scientific Affairs Board (PSAB) issued a statement on the Centers for Disease Control and Prevention (CDC) new Interim Guidance on Monitoring and Movement of Persons with Suspected Ebola Contact. While quarantine is primarily a state-by-state responsibility, there is a need for a coherent national policy. CDC has quarantine authority for interstate movement of persons who pose a public health risk; states have authority within their own borders. The CDC guidelines are based on current scientific and medical evidence that Ebola is transmitted only by direct contact with body fluids from someone who is symptomatic, i.e., onset of fever, fatigue, muscle pain, headache, sore throat, followed by vomiting, diarrhea, rash and, in some cases, both internal and external bleeding. The guidelines provide additional layers of protection against the inadvertent spread of Ebola within the United States since isolation and restricted travel of asymptomatic individuals with suspected high-risk Ebola contact provides adequate public health protection. The ASM statement is available at http://www.asm.org/index.php/news-room/asm-press-releases/93-policy/93236-ebola-10-29-14.

On 7 November, PSAB sent a letter to Congress supporting the Administration’s $6.18 billion emergency funding request to combat the Ebola virus outbreak. The request includes $4.64 billion for immediate needs and $1.54 billion in contingency funding to ensure that there are resources available to respond to the evolving epidemic both domestically and internationally. A bulk e-mail was sent to ASM members informing them of the letter and providing background information on the funding request, the ASM’s updated Ebola Resources Page, and a newly posted Ebola FAQ. The ASM Ebola Resources page is available at http://www.asm.org/index.php/newsroom/asm-press-releases/89-newsroom/media-info/93111-asm-ebola-resources.

ASM Testifies before the Federal Experts Security Advisory Panel Biosafety and Biosecurity Issues

On 27 October, Ronald Atlas, Chair of the ASM Public and Scientific Affairs Board (PSAB), testified at the request of the Federal Experts Security Advisory Panel (FESAP). To strengthen USG oversight for work with infectious agents, including (but not limited to) Biological Select Agents and Toxins (BSAT), the FESAP has undertaken a comprehensive federal review that will result in specific recommendations to strengthen the government’s biosafety and biosecurity practices and oversight system for federally funded activities, consistent with the need to realize the public health and security benefits of such work. The ASM addressed the following FESAP tasks in its testimony: (i) identify needs and gaps and make recommendations to optimize biosafety, biosecurity, oversight, and inventory management and control for BSAT; (ii) identify actions and any regulatory changes necessary to improve biosafety and biosecurity; (iii) identify an approach to determine the appropriate number of high-containment U.S. laboratories required to possess, use, or transfer BSAT. The PSAB presentation is posted at http://www.asm.org/ASM-biosafety-biosecurity.

Gain-of-Function Research

The White House Office of Science and Technology Policy on October 17 announced that the U.S. government will undertake a deliberative process to assess the risks and benefits of certain gain-of-function (GOF) experiments with influenza, SARS, and MERS viruses in order to develop a new Federal policy regarding the funding of this research. ASM sent out an alert to members informing them of the process, a funding pause for GOF research with links to background information. The alert is available on the ASM Public Policy website at https://www.asm.org/index.php/public-policy/98-policy/issues/93225-usg-gof10-20.

ASM Meetings with Policy Makers

On 22 October, Ronald Atlas, Chair of the ASM Public and Scientific Affairs Board (PSAB), ASM President Timothy Donohue, and Janet Shoemaker met with officials at the Office of Scien
ence and Technology Policy about Ebola virus issues and Gain-of-Function (GOF) research policy. The federal government recently paused funding for certain GOF experiments on select viruses. A statement from Francis S. Collins, Director of the National Institutes of Health is available at http://www.nih.gov/about/director/10172014_statement_gof.htm.

On 24 October, Ronald Atlas; Kenneth I. Berns, chair, PSAB Committee on Biomedical Research; Gail Cassell, chair of the PSAB Committee on Biomedical Research; and Janet Shoemaker visited Fort Detrick to meet with officials from the U.S. Army Medical Research and Materiel Command to discuss medical microbiology activities at USAMRIID.

On 21 October, Kimberly Walker, Manager, Public Affairs, attended a meeting at the White House with Sarah Charles, NSC Director for Development and Humanitarian Affairs; Nancy Lindborg, Assistant Administrator, United States Agency for International Development; and Nancy Abella, NSC Director for Immigration and Visa Security to discuss the Obama administration’s efforts to combat and treat Ebola in West Africa, and on developments in connection with the domestic response.

On 23 October, the White House Office of Science and Technology Policy (OSTP) held a conference call with stakeholders to discuss steps the administration is taking in response to the Ebola crisis and the scientific community’s role in response efforts. On the call were Jo Handelsman, Assistant Director for Science, Office of Science and Technology Policy and former ASM President; Beth Bell, Director, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Centers for Disease Control and Prevention; Arjun Srinivasan, Associate Director for Healthcare Associated Infection Prevention Programs, NCEZID; and Wendy Taylor, Director, Center for Accelerating Innovation and Impact, U.S. Agency for International Development. Kimberly Walker participated in the conference call.

**ASM Staff Attend Congressional Hearings on Ebola Response**

ASM Staff attended a number of Congressional hearings covering the national and global response to Ebola virus disease in the West African region comprising Guinea, Sierra Leone and Liberia, and the response in the United States. The 12 November Senate Appropriations Committee hearing “U.S. Government Response: Fighting Ebola and Protecting America” heard testimony from witnesses Sylvia Mathews Burwell, Secretary, U.S. Department of Health and Human Services; Jeh Johnson, Secretary, U.S. Department of Homeland Security; Heather Higginbottom, Deputy Secretary, Management and Resources, U.S. Department of State; and others. Outside Witness Testimony was placed in the record from more than 50 groups, including the American Public Health Association, the Association for Professionals in Infection Control and Epidemiology, the Infectious Diseases Society of America, the National Coalition of STD Directors, the University of Maryland, Baltimore, and the University of Nebraska Medical Center. To view the archived hearing in its entirety, please click http://energycommerce.house.gov/hearing/examining-us-public-health-response-ebola-outbreak.

**Congressional News**

Following the November election, the makeup of the Republican-controlled U.S. House of Representatives stands at 244 Republicans to 184 Democrats, with several elections still undecided as of mid-November. The Senate stands at 52 Republicans, 44 Democrats, and 2 Independents, with two states not determined as of mid-November.

**ASM Attends CLIAC Meeting**

ASM staff attended the Clinical Laboratory Improvement Advisory Committee (CLIAC) meeting on 6 November. This meeting included updates from the Food & Drug Administration (FDA), the Centers for Disease Control and Prevention (CDC), and the Centers for Medicare and Medicaid Services (CMS). Alberto Gutierrez, director of the Office of In Vitro Diagnostics and Radiological Health at the FDA Center for Devices and Radiological Health announced a January 2015 meeting with stakeholders to discuss
concerns about the FDA draft guidance on laboratory-developed tests (LDTs). To read more about the other topics covered in the November meeting, please see http://wwwn.cdc.gov/cliac/.

ASM Staff Attends JHU Public Health Workshop on Ebola

On 14 October, the “Dean’s Symposium on Ebola: Crisis, Context and Response” was held at the Johns Hopkins Bloomberg School of Public Health. Speakers included Michael Osterholm, Center for Infectious Disease Research and Policy, University of Minnesota; Peter Jahrling, National Institute for Allergy and Infectious Diseases Integrated Research Facility; Joshua Sharfstein, Maryland Secretary of Health & Mental Hygiene; and Nancy Kass, Deputy Director for Public Health, Berman Institute of Bioethics, Johns Hopkins University. Event videos are available online at http://www.jhsph.edu/events/2014/ebola-forum/.

Education Board

JMBE Debuts First Thematic Issue on Scientific Ethics

The editors of ASM’s Journal of Microbiology & Biology Education (JMBE)—the premier journal for microbiology and biology education research—are pleased to announce the publication of the December 2014 issue (volume 15, issue 2) of the journal.

The issue is introduced by an editorial from JMBE editor-in-chief Samantha L. Elliott, Ph.D., an associate professor of biology at St. Mary’s College of Maryland. The editorial highlights several endeavors JMBE has embarked on since Elliott assumed leadership of the journal in January 2014. One significant endeavor is the launch of a thematic section, unveiled in this new issue. The inaugural section will focus on scientific ethics “as a broadly-applicable topic that is highly relevant, yet often underserved in our discipline,” says Elliott. Led by guest editors Beth A. Fischer, Ph.D., and Michael J. Zigmond, Ph.D., from the University of Pittsburgh, and Frederick Grinnell, Ph.D., from UT Southwestern Medical Center, the section contains a collection of essays by recognized leaders in the responsible conduct of research (RCR). The collection is divided into five categories: (i) RCR education; (ii) ethics in science education; (iii) the research community and matters of scientific publishing, authorship, and conflicts of interest; (iv) perspectives on research integrity; and (v) innovative ways to introduce ethics into STEM education.

Another important update made by the journal involves an effort to encourage the adoption of safe practices when microbes are used in teaching laboratories. Going forward, each JMBE manuscript is required to directly address the ASM Laboratory Safety Guidelines if microbes are used in activities described in the manuscript. “By directly explaining these safety concerns, we teach others the best practices in adopting our activities, and reaffirm our commitment to student and instructor safety,” says Elliott.

Other JMBE updates include offering author guidelines in Spanish and Mandarin and preparing a document that explains considerations of institutional review board consent in biology education research. The journal has also launched a “JMBE Profiles” interview series designed to help readers and authors “meet the people behind JMBE, learn about writing for a scientific education journal, and hear discussions about current issues in biology education,” says Elliott. The first two episodes are available at ASM’s YouTube page: www.youtube.com/MicrobeWorld.

To read the full issue, including articles from the journal’s Research, Curriculum, and Perspectives sections, visit http://jmbe.asm.org.

JMBE articles promote good pedagogy and design, foster scholarly teaching, and advance biology education research. The journal’s editors welcome article submissions, and manuscripts are accepted and reviewed on a rolling basis. Sign up for JMBE news and eTOC alerts by visiting http://jmbe.asm.org/index.php/jmbe/user/register.

ASMCUE 2015: Build Your Knowledge, Expand Your Network

José Antonio Bowen, Ph.D., author of Teaching Naked: How Moving Technology Out of Your College Classroom Will Improve Student Learning, will open the 2015 Annual ASM Conference for Undergraduate Educators (ASMCUE) on 28–31 May. Bowen, a national award-winning educator and the newly appointed president of Goucher College, is a pioneer in educational technology and active learning, two topics covered extensively at ASMCUE. Held at the Renaissance Austin Hotel in Austin, Tex., ASMCUE 2015 will offer four days of intensive professional development sessions and inspiring talks by leaders at the forefront of science and teaching.

On this occasion, ASMCUE’s 22nd year, the conference will include plenary lectures on “The Ebola Virus,” by Erica Ollmann Saphire, Ph.D. (The Scripps Research Institute), and “Microbes and Spaceflight,” by Duane Pierson, Ph.D. (NASA Johnson Space Center). In addition, there will be a special presentation from an Apple, Inc., representative, along with numerous discussions of advances in STEM education and research, lab safety guidelines, teaching tools, student learning, and more!

Join ASMCUE 2015 to (i) attend sessions on the latest topics in biology and science education; (ii) present your work and get feedback from other faculty and researchers; (iii) connect with friends, old and new, at multiple networking sessions; (iv) explore the amazing city of Austin; and (v) be motivated and inspired for months to come.
Other highlights include concurrent sessions on classroom-tested resources for advancing in pedagogy, scholarship, research design, methodology, and assessment, along with microbrew sessions full of ideas and thoughts on best practices in microbiology and biology education. You’ll have several opportunities to see poster presentations, network with colleagues, and visit with higher education vendors.

Conference registration is available online at a discounted rate from 1 January to 9 March, and the abstract submission deadlines are 2 February for poster presentations and 9 February for oral “Microbrew” presentations. The conference program will be posted at http://www.asmcue.org in December 2014.

Check out ASMCUE 2015 on Facebook at https://www.facebook.com/groups/asmcue or follow conference updates on Twitter at @asmkelly.

International Affairs

Partnerships for Point-of Care Testing in Ukraine

To meet global need, the pipeline for new HIV point-of-care (POC) diagnostics continues to grow. Technologies such as the Alere Pima™ Analyser and its corresponding CD4 test can make a major impact on patient management by providing same-day CD4 T-cell counts, which are used to start antiretroviral treatment (ART) in HIV-positive individuals and monitor treatment outcomes. When used by properly trained staff, this POC test can not only reduce the time to ART initiation, but provide access to CD4 monitoring at sites that have not benefited from other more complex CD4 platforms.

Through a collaboration with the U.S. Centers for Disease Control and Prevention-Ukraine, ASM is supporting the International HIV/AIDS Alliance in Ukraine (Alliance) with plans to implement the Alere Pima™ Analyser. Alliance currently conducts large-scale HIV testing in Ukraine, especially for most at risk populations (MARPs) and hopes that CD4 detection provided by this technology will allow early and timely commencement of ART and monitoring of patients’ health condition.

In September 2014, ASM jointly conducted two workshops on the use of the Pima Analyser with capillary blood sampling for medical personnel of Ukrainian regional HIV/AIDS centers and local nongovernmental (NGO) voluntary counseling and testing sites. ASM consultant Mark Pettigrove has been providing technical assistance for strengthening HIV diagnostic services in Ukraine for the past year and co-facilitated the workshop. The training, which included lecture and demonstration, provided background on CD4 hematology and laboratory, introduced the concept of POC testing, reviewed technical aspects of the analyser, and discussed results from studies on the implementation of the analysers in resource-constrained settings. From his perspective, “The participants were enthusiastic during the workshop and recognized the potential of the POC analyser. The regional HIV/AIDS NGOs under Alliance are the link between MARPs and health care services. Though there is unrest in the eastern part of the country, people are doing their best to continue providing valuable public service.”

Center for the History of Microbiology/ASM Archives

Ron Atlas Collection at the Center for the History of Microbiology/ASM Archives

I was recently asked to assist in the accessioning of the newly established Ron Atlas Collection in the Biological Warfare Collection of the American Society for Microbiology’s (ASM) Center for the History of Microbiology/ASM Archives (CHOMA). Even at this preliminary stage of processing, it’s clear that the Atlas Collection offers a glance into the mind of one of America’s leading thinkers on biosecurity during perhaps the most volatile era at the intersection of government and the life sciences in recent times: the post-9/11 and “anthrax letter” years. Atlas of course was President of ASM in 2003, respected by scientific and technical professionals, policymakers, and the lay public alike for his clear and straightforward communication of the critical biosecurity issues that consumed the nation during his tenure. I would qualify myself as fitting most closely into the last of those categories at the time, having just completed my postdoc in chemical and biological weapons nonproliferation in 2002 and only beginning to grasp the importance of what was happening all around me. Now, looking back through the eyes of Dr. Atlas, what I find most intriguing are his handwritten notes and underlines as the events that altered the field of microbiology unfolded on paper before him.

The artifacts of the Atlas Collection span decades but primarily fall within a 10-year range, beginning with President Clinton’s growing focus on bioterrorism in 1998 and waning with the Iraq War decline in 2007. As faxes and FBIS searches give way to e-mails and website printouts, we see events that range from the Clinton administration push for and subsequent failure of a Biological and Toxin Weapons Convention (BWC) verification protocol and warnings of threats of weapons of mass destruction (WMD) terrorism in the 1990s, to the events of 9/11 and the anthrax letters, and the wars since 2001 and federal and public health responses to all of these events. Some of the more recent issues covered include the implementation of...
Obituaries

Edwin E. Geldreich

Edwin E. Geldreich, Jr., a long time member of ASM and a Fellow of the American Academy of Microbiology, died at age 92 on 7 October 2014. A native Cincinnatian, he received both his undergraduate and masters’ degrees in biological sciences from the University of Cincinnati. He served in the U.S. Army in the European campaign during the Second World War. Initially hired by the noted bacteriologist C. T. Butterfield, his entire professional career was spent with various federal agencies working on water-related programs. He was a charter employee of the U.S. Environmental Protection Agency (EPA), where he served as both Chief Microbiologist and Senior Advisor for drinking water research activities.

He was the author of numerous peer-reviewed scientific research articles and other publications, including the classic *Handbook for Evaluating Water Bacteriological Laboratories.* The recipient of EPA Bronze and Silver medals, he also received numerous other awards, including the Kimble Medal of ASM. In 1989 he was a recipient of the Abel Wolman Award of Excellence from the American Water Works Association. In 1991 he was the Allen Hazen Lecturer to the New England Water Works Association. Educational activities included serving as a consultant for the World Health Organization dealing with water-related issues in Caribbean and Latin American countries. He was held in high esteem among his federal colleagues and by others both in academia and in industry for his numerous contributions to the field of drinking microbiology. He was noted for providing encouragement to young scientists to pursue their research interests aimed at improving water quality.

Ed had many avocational interests including photography, travel, playing the organ, and gardening. He was also a licensed ham radio operator who built much of his own equipment. He was preceded in death by his wife Detta, to whom he was married for over 55 years. He is survived by two daughters, Linda Lambers and Pamela Bogosian, their respective spouses, and four grandchildren.

Eugene W. Rice
Terry C. Covert
Martin J. Allen

David Gibson

David T. Gibson, Professor Emeritus of Microbiology at the University of Iowa, passed away on 24 July 2014 at the age of 76. Dave was a beloved husband, father, and grandfather, a gifted scientist, teacher and lecturer, and a dear friend to so many. Gibson was born in Wakefield in 1938, and spent his early years in Redcar, on the northeast coast of Yorkshire. He emigrated with his wife Janet to the United States in 1964 after receiving his B.Sc. and Ph.D. degrees in biochemistry at The University of Leeds. In 1967, after post-doctoral studies with Charles Sih at the University of Wisconsin and Reino Kallio at the University of Illinois, he joined the faculty of the Department of Microbiology at The University of Texas at Austin. The following year he returned to England and was employed as a research scientist at the Pharmaceuticals Division of Imperial Chemical Industries. In 1969 he returned to The University of Texas, eventually rising to the positions of Professor and Director of The Center for Applied Microbiology. In 1988 he moved to The University of Iowa to take the first endowed Edwin B. Green Chair in Biocatalysis and Microbiology, a position he held until his retirement in 2004.

Gibson’s research focused on the pathways used by microorganisms to degrade aromatic hydrocarbons and environmental pollutants. His work at the University of Texas focused on the mechanisms involved in the activation...
of molecular oxygen and the hydroxylation reactions leading to preparation for fission of the aromatic nucleus. This pioneering work led to his discovery of unique stereospecific reactions and enzymes used by bacteria to add dioxygen to benzenoid molecules ranging in size from benzene to benzo[α]pyrene. Novel bacterial metabolites that were isolated and identified in his laboratory have since been used to synthesize important biologically active compounds. Gibson’s work at the University of Iowa included the determination of the first structures by X-ray crystallography of the multicomponent dioxygenase enzyme systems used by bacteria to initiate the biodegradation of aromatic hydrocarbons. Gibson’s work, chronicled in more than 200 publications, provided the scientific foundations for the development of the fields of bioremediation and biocatalysis.

Gibson served on the editorial boards of the Journal of Bacteriology and the Journal of Biological Chemistry and was the recipient of numerous awards. In 1976 he was the American Academy of Microbiology Latin America Visiting Professor at the National Polytechnic Institute in Mexico City, and in 1983 he was recipient of the Piper-Stevens Award for Teaching and Research. Gibson was elected as a Fellow in the American Academy of Microbiology in 1983 and as a Fellow in the American Association for the Advancement of Science in 1994. In 1996, ASM honored Gibson by devoting a symposium to his work at the annual meeting in New Orleans. The following year he received the ASM Procter and Gamble Award in Applied and Environmental Microbiology. He was elected to The National Academy of Sciences in 2004.

Gibson was a dedicated professor of microbiology for over 34 years. He was known as a kind but demanding professor with extremely high standards. Dave’s 27 graduate students, 30 postdoctoral fellows, and numerous visiting scientists became an extended and international “scientific family” of microbiologists, chemists, and biochemists, many of whom gathered at a memorial celebrating Dave’s life and accomplishments on 13 September 2014 at the Tower Hill Botanic Garden in Boylston, Mass., where a tree was dedicated in his honor. He is survived by his wife Janet, whom he married in 1963; daughters Karen Gibson and Christine Ruddy; son-in-law Kevin Ruddy; grandchildren Elizabeth, Nathan, and Nicholas; and brothers John and Philip.

Karen Gibson
Sol Resnick
Rebecca Parales

Wilhelmus Nicolaas Konings
On 5 July 2014, Wilhelmus Nicolaas Konings (known as Wil), Emeritus Professor of Molecular Microbiology at the University of Groningen in the Netherlands, passed away unexpectedly at the age of 77. Konings received his Ph.D. from the University of Groningen in 1969. From 1969 to 1971 he worked at the National Institutes of Health (NIH), in Bethesda, Md., as a postdoctoral fellow. In 1971, he was appointed Lecturer at the University of Groningen, and in 1980, he became Professor of Microbiology. In 2002, he retired as Professor Emeritus. In 2001, he served as Professor Emeritus. In 2002, he retired as Professor Emeritus, leaving a legacy of more than 440 scientific papers and 7 patents, as well as numerous students and postdoctoral scientists who were trained in his laboratory. After retiring from the University of Groningen, he became associated with the University of Stellenbosch in South Africa, where he lectured for more than 10 years.

From the mid-1970s, he played a prominent role in the field of microbiology, especially in membrane biology. He was an outstanding researcher with an international reputation and stature in the rich tradition of Dutch microbiology. Scientifically, he will be remembered best for his extensive work on substrate transport in bacteria and archaea. Konings developed an interest in bacterial transport while doing postdoctoral research in the laboratory of Ernst Freese at the NIH, where he was studying sporulation in Bacillus subtilis. Meeting Ron Kaback influenced him to study transport in B. subtilis vesicles rather than sporulation. Shortly thereafter, he discovered how to energize transport with an artificial electron donor system, which allowed the generalization of the vesicle system to many bacteria in addition to Escherichia coli. As a result of these and many more experiments, the two became lifelong friends and scientific colleagues.

In 1980, Wil proposed a model of energy recycling by product secretion, where a transporter catalyzes efflux of metabolic end products and thus conserves metabolic energy. Other work included the identification of specific antiprot systems that play a role in energy conservation as part of simple metabolic pathways. Further hallmarks of his work are discoveries on the regulatory effects of intracellular pH and redox potential on the activity of transport proteins. His research on amino acid and peptide transport and the proteolytic system of lactic acid bacteria initiated intense contacts with the dairy industry and the organization of a European network on lactic acid bacteria. After his retirement, Wil continued to work as cofounder of the Biotechnology company IMENZ Bioengineering.

Central to Konings’ work was the use of well-defined model systems, such as isolated cytoplasmic membrane vesicles, which could be fused with liposomes reconstituted with an energy-generating source such as cytochrome c oxidase. These systems were used to study transport processes with membranes derived from strictly anaerobic bacteria and plasma membranes from yeasts and fungi. Later, he employed liposomes in which purified transport proteins were embedded in a functional state, including the functional reconstitution of membrane
proteins into liposomes composed of tetra-ether lipids isolated from extremophilic archaea. In the community of extremophilic research, Konings is best known for his contributions on how microbes adapt the lipid composition of the cytoplasmic membrane to extreme conditions and how cells deal with an increased ion permeability at elevated temperatures.

Another highlight is his work on bacterial multidrug resistance transporters involved in the secretion of a wide variety of unrelated toxic compounds, including antibiotics from the cell. He identified a bacterial multidrug transporter that is a structural and functional homolog of human P-glycoprotein that plays an important role in the resistance of cancer cells to cytotoxic drugs. He discovered that lipophilic substrates of multidrug transporters are transported from the inner layer of the cytoplasmic membrane into the extracellular milieu.

In 1997, Wil Konings was elected to membership in the Royal Dutch Academy of Art and Sciences (KNAW). In 2001, he was knighted by Queen Beatrix in the Order of the Dutch Lion. We will remember Wil Konings as an important, versatile and passionate scientist who inspired many young researchers. He will always remain among our dearest memories as a wonderful scientist and a great friend and close colleague. Wil is survived by his wife of over 50 years, Ine Konings-Stolte, a lawyer, and three children, Karen (a cardiologist), Wouter (an industrial designer) and Lili (a psychiatric social worker) and eight grandchildren.

A. J. M. Driessen
B. Poolman
University of Groningen
Groningen, the Netherlands

H. R. Kaback
University of California, Los Angeles

J. E. Walker
MRC Mitochondrial Biology Unit
Cambridge, United Kingdom
Reviews and Resources

BOOK

Cases in Medical Microbiology and Infectious Diseases, 4th ed.

Infectious diseases continue to be among the leading causes of illness, disability, and death in the United States and worldwide. The microbial diseases that physicians encounter, as well as the tools to diagnose and manage infections, have changed significantly in recent years. Advances in diagnostics, particularly nucleic acid-based assays, enable more rapid and specific testing; yet, the rise of multidrug-resistant pathogens has reduced the physician’s arsenal of treatments. More vaccines are available to prevent known infections, while outbreaks of emerging pathogens continue to present new challenges. In this evolving landscape of infectious disease, thorough and up-to-date texts are invaluable resources for those training in the health professions.

The textbook Cases in Medical Microbiology and Infectious Diseases aims to provide a working knowledge of infectious diseases in different systems of the human body; the content is presented through detailed clinical cases that emphasize differential diagnosis among various possible microbial etiologies. The book is a product of three multidisciplinary authors who are undisputed experts in the fields of clinical microbiology and infectious diseases. This book is an excellent resource for medical students, especially those taking courses related to microbiology and infectious diseases or preparing for board examinations, as well as medical residents and clinical microbiologists. Given that the book includes several cases which have a global public health impact, this is a valuable resource for students of clinical microbiology and infectious diseases from all over the world.

The book begins with an up-to-date review of various diagnostic approaches, including state-of-the-art molecular techniques used in the clinical microbiology laboratory. Following the diagnostic section, this 4th edition reviews 74 clinical cases, 42 of which are new since the 3rd edition. The 32 cases retained from earlier editions have been thoroughly updated with the latest available information on the diseases and pathogens. Each clinical case focuses on several key areas, including the organism’s characteristics and laboratory diagnosis, pathogenesis and clinical characteristics of infection, epidemiology, prevention, and, in some cases, drug resistance and treatment. The cases are grouped into seven sections, with the first six sections focusing on diseases of a specific organ system. Each section has an excellent introduction outlining the types of infections and considerations important for that organ system and a table of commonly encountered pathogens of the system. A major attraction of the fourth edition is the inclusion of a new section called “Advanced Cases,” which discusses case presentations of newly recognized and complex diseases. Although this section replaces the “Emerging and Re-emerging Diseases” section from the third edition, significant emerging infections are incorporated in new cases throughout the book. A large number of color photographs support the case discussions. For easy reference, the textbook includes a table of normal laboratory values and a glossary of medical terms. All chapters and case discussions are well referenced, with citations through 2013.

The book is exceptionally well written and remarkably comprehensive, covering a wide array of medically significant bacteria, viruses, fungi, and parasites. One strength of the book as an educational tool is the layout of the cases. Each case begins with the clinical description and questions that lead the reader through a thorough investigation of the clinical case, possible causes, and management options. Following each set of questions is the comprehensive case discussion; through these discussions, the authors do a great job of addressing current topics important to the field, including antimicrobial resistance, molecular techniques for diagnosing infectious diseases, the effects of the microbiota on human health, the impact of public health measures on disease outcome, and the role of vaccines and monoclonal antibodies in the prevention and treatment of infectious diseases. Included discussions on the global impact of infectious diseases are timely and highly relevant, given the globalization of trade and tourism. To facilitate review of the cases, it would have been helpful to include a boxed summary after each case discussion highlighting the disease, pathogen, and chief points of the case. Of course, a major strength of this book lies in the expertise of the authors in their respective fields; the level of detail and well-thought-out nature of the text speaks to the diligence that the authors have put together in compiling this text.

In summary, this textbook is a ready
reference for anyone desiring a well-illustrated, state-of-the-art, and easy-to-read textbook on infectious diseases and is a serious candidate for departmental, hospital, and university libraries. Many advances have occurred in the field of microbiology and infectious diseases over the past decade, and the timely update of this fourth edition is a welcome resource from experts in the field who have done a commendable job covering the exciting and ever-changing field of infectious diseases.

Dharanesh Gangaiah
Margaret E. Bauer
Indiana University School of Medicine,
Indianapolis

Join ASM Speakers’ Bureau!

Interested in sharing your microbiology or immunology career experience with ASM Student Chapters?

ASM is looking for members employed in the clinical lab or industry, such as —

- Bioremediation
- Biosafety
- Biotechnology
- Dairy
- Food Safety
- Indoor Air Quality
- Medical Device
- Pharmaceuticals
- Policy
- Public Health
- Tech Sales
- Water Quality

The Speakers’ Bureau is designed to attract students to microbiology career opportunities. Speakers share with ASM Student Chapters information about their careers, how they got started and tips for success. Speakers can give a live, recorded or video call presentation!

ASM Members can visit [http://bit.ly/ASMspeakers](http://bit.ly/ASMspeakers) to learn more and sign up!
Application Deadlines

Mäkelä – Cassell Travel Award for Early Career Scientists. Named in honor of ASM past president Dr. Gail Cassell and FEMS past president Dr. Helena Mäkelä, this award is offered jointly by ASM and the Federation of European Microbiology Societies (FEMS) to support the exchange of one member from each organization to present a poster of their research at the other society’s main conference.
Deadline: 31 January 2015.

National Registry of Certified Microbiologists (NRCM) Certification. The NRCM certifies microbiologists at the prebaccalaureate/baccalaureate, master’s, and doctoral-levels. Certification is offered in biological safety; food safety and quality; and pharmaceutical and medical device. NRCM certification is achieved by passing an online multiple-choice exam that is offered daily in the month of April at testing centers worldwide.
WWW: www.asm.org/nrcm
Deadline: 1 February 2015.

ASM Undergraduate Research Fellowship. The ASM Undergraduate Research Fellowship program provides undergraduates interested in graduate careers (Ph.D. or M.D./Ph.D.) in microbiology with opportunities to conduct full-time research at their home institutions and present research results. Fellows spend at least 10 weeks in the summer conducting research under the direction of ASM member faculty mentors. In addition, dependent on abstract submission and acceptance, fellows are supported to present their research results at the following summer’s ASM General Meeting. Note: This program requires a joint application from the student and an ASM member willing to serve as the faculty mentor for the duration of the fellowship.
WWW: http://www.asm.org/urf
Deadline: 1 February 2015.

Biology Scholars Program. Developed with support from NSF (grant no. DUE-1022542), the ASM-NSF Biology Scholars Program offers three professional development residencies that help biology and microbiology faculty in taking their classroom teaching and educational research to the next level. Each residency is a yearlong commitment comprised of online assignments, one or two face-to-face meetings, and specialized peer-to-peer mentoring. Assessment Residency participants learn to measure students’ foundational knowledge, monitor gains in learning, and collaborate with colleagues to improve student learning and performance. Research Residency participants design research projects that investigate student learning; understand methods for collecting, analyzing, and interpreting surveys and data on student learning outcomes; and identify venues for publishing study results. Transitions Residency participants “transition” from conducting scholarly work in student learning to publishing in biology and/or science education venues.
WWW: http://www.biologyscholars.org
Deadline: 1 February 2015.

ASM-CDC Postdoctoral Research Fellowship. Postdoctoral scientists are encouraged to submit applications for 2014 ASM-CDC Postdoctoral Research Fellowships. Offered through the ASM-CDC Program in Infectious Disease and Public Health Microbiology, each fellowship is a two-year research experience under the mentorship of CDC scientists. The goal of the fellowship is to support the development of new approaches, methodologies, and knowledge in infectious disease prevention and control in areas within the public health mission of the CDC. All fellows perform research at a CDC location. Available fields of study include bacterial and mycotic diseases, viral and rickettsial infections, and HIV/AIDS.
WWW: http://www.asm.org/cdcfellowship
Deadline: 15 February 2015.

American Board of Medical Microbiology (ABMM) Certification. Certifies the expertise of doctoral-level microbiologists seeking to direct public health or clinical microbiology laboratories. ABMM certification is achieved by passing an online multiple-choice exam that is offered daily in the month of June at testing centers worldwide.
WWW: www.asm.org/abmm
Deadline: 1 April 2015.

About Application Deadlines

The Application Deadlines section provides ASM members with information about certification programs, awards, and fellowships sponsored by ASM. More resources are available to members on the website at http://www.asm.org/index.php/awards-grants-whats-new-in-asm-awards-grants-fellowships-and-professorships.html. The website provides direct links to program Web pages for complete details, including eligibility requirements and application information.
ASM Meetings Calendar

9–11 February 2015.
ASM Biodefense and Emerging Diseases Research Meeting.
Washington, D.C.
WWW, http://www.asmbiodefense.org/

13–16 March 2015.
Mechanisms of Interbacterial Cooperation and Competition.
Washington, D.C.
WWW, http://conferences.asm.org/

26–29 April 2015.
31st Clinical Virology Symposium.
Daytona Beach, Fla.
WWW, http://www.clinicalvirologysymposium.org/

8–11 May 2015.
Antimicrobial Resistance in Zoonotic Bacteria and Foodborne Pathogens.
WWW, http://conferences.asm.org/

ASM Conference for Undergraduate Educators.
Austin, Tex.
WWW, http://www.asmcue.org/

30 May–2 June 2015.
ASM General Meeting.
New Orleans, La.
WWW, http://gm.asm.org/

12–16 June 2015.
Prokaryotic Cell Biology and Development.
WWW, http://conferences.asm.org/

8–12 September 2015.
ASM Conference on Pseudomonas 2015.
Washington, D.C.
WWW, http://conferences.asm.org/

1st ASM Conference on Rapid Next-Generation Sequencing and Bioinformatic Pipelines for Enhanced Molecular Epidemiologic Investigation of Pathogens.
Washington, D.C.
WWW, http://conferences.asm.org/

24–29 October 2015.
7th ASM Conference on Biofilms.
Chicago, Ill.
WWW, http://conferences.asm.org/

2–5 November 2015.
Chicago, Ill.
WWW, http://conferences.asm.org/

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/events-calendar/cat.listevents/2012/01/18/. 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 at https://info.asm.org/index.php/events-calendar/icaevent.edit/0?year=2012&month=01&day=18.
The Department of Microbiology & Molecular Genetics at Michigan State University seeks candidates at the Assistant or Associate Professor level for a position in the microbial ecology of infectious disease including interactions between host, microbiome, pathogen and environment. Of special interest within the broad area of the microbial ecology of infectious disease are systems biology and evolution at the molecular level, physiology and metabolic networks of microbial consortia, interactions of pathogens with mammalian hosts, insect vectors, and/or microbiota within them, host innate immunity to microbes, and the application of “omics” technology to study these areas. Significant opportunities exist for collaboration with faculty associated with the NSF BEACON Center (Evolution in Action), the NIH Enterics Research Investigational Network, the NIH International Center of Excellence for Malaria Research, the RDP, and the Center for Water Sciences. A strong record of research accomplishment and/or potential for an independent, externally funded research program with national visibility is expected. Academic rank will be commensurate with experience. Teaching in graduate, professional, and/or undergraduate programs is expected. The offer will include a competitive startup package and laboratory facility.

Applications should be submitted electronically at https://jobs.msu.edu (posting #0519) with requisite material (letter of interest, curriculum vitae listing past and current funding if appropriate, a statement of future research goals, and names of three potential references (not to be contacted until approval is received from the applicant)) uploaded as a single PDF. Review of applications will begin immediately and remain open until filled.

Additional information could be obtained via email to the Search Committee Executive Assistant at mmgchair@msu.edu. http://www.mmg.msu.edu

MSU is an affirmative-action, equal-opportunity employer and is committed to achieving excellence through diversity. The University actively encourages applications of women, persons of color, veterans, and persons with disabilities, and we endeavor to facilitate employment assistance to spouses or partners of candidates for faculty and academic staff positions.
Small Things Considered

The Cold Side of Microbial Life
http://schaechter.asmblog.org/schaechter/2014/03/the-cold-side-of-microbial-life.html
by Gemma Reguera

The almost permanent frozen state of many northern soils—the permafrost—limits microbial activity and the turnover of organic matter, contributing to the storage of approximately 25% of all of the planet’s soil organic matter. But do not be fooled by the frozen state: the permafrost harbors a great diversity of metabolically active microbes. Just how active, we don’t know. As global warming continues to thaw large areas of permafrost, the activity of these microbes could be stimulated further, promoting the turnover of the organic matter trapped in the soils and the release of greenhouse gases into the atmosphere.

But how active are permafrost microbes? Microbial ecologists have many techniques to profile microbial communities and assess the abundance of specific phylogenetic groups. However, measuring the metabolic activity of specific phylogenetic groups and their contribution to carbon cycling and biomineralization is more challenging. In a recent paper, Tuorto et al. combined two powerful techniques to address both. They first used stable isotope probing with $^{13}$C to selectively label the DNA from metabolically active permafrost cells when they incubated permafrost soil microcosms with $^{13}$C-acetate at temperatures ranging from $0^\circ$ to $-20^\circ$ C. The metabolically active, growing microbes incorporated $^{13}$C isotope into their newly replicated DNA, which could be separated from the original, lighter $^{12}$C-DNA of the nongrowing population. They then profiled the active and inactive microbial communities with techniques based on the 16S rDNA sequences present in the heavy and light DNA pools, respectively.

The profile of the permafrost communities revealed a great diversity of resident bacteria: 152 operational taxonomic units (OTUs). Most of them incorporated the $^{13}$C isotope; hence, they were able to grow with acetate (or byproducts of its metabolism) at subzero temperatures. Furthermore, the permafrost community was active across a range of temperatures, but half of the active OTUs had very specific temperature preferences. Interestingly, the groups with a temperature preference contained the same phyla ($\text{Acidobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes and Proteobacteria}$); however, the types of active microbes within each phylum varied: these microbes had specialized their metabolism to grow within a narrow range of temperatures.

How could a microbe specialize to grow at a specific subzero temperature? Most likely, it has evolved strategies to meet its water needs at that particular temperature. In frozen soils, water is restricted to a very thin, salty layer of liquid surrounding the soil particles. The high concentration of solutes in this liquid layer prevents the water from freezing, but to access this water resource microbes need to evolve mechanisms for both cold- and salt-adaptation. Some microbes, for example, synthesize extracellular polymeric substances (EPS) as cryoprotectants, but also to facilitate attachment to soil particles and promote water retention and nutrient absorption. In fact, increases in EPS synthesis and bacterial abundance in cold ecosystems correlate well with decreases in temperature. Changes in fatty acid composition have also been reported for these microbes, which modulate the stability and functioning of the lipid membrane to the low water activity in their surroundings.

Results from this study support the notion that the metabolic activity of permafrost communities is resilient to temperature variations. Although many of the metabolically active microbes were active within a narrow range of temperatures, the microbial community fluctuated with the changing temperatures to maintain its overall productivity. Hence, it is reasonable to think that they could continue to be active, and even be stimulated, if global temperatures continue to rise and permafrost soils continue to melt. These conditions may also “wake up” dormant microbes stimulating carbon turnover and gas emissions from these ecosystems in unpredictable ways. Given that permafrost soils occupy ~20% of the Earth’s land surface, changes in the activity of resident microbes are likely to impact the planet globally. Despite these being educated guesses, it is clear that permafrost microbial activities need to be accounted for in global climate models to improve their predictive value. But the real message here is that this is indeed a planet of microbes. Microbes are active everywhere we look, even in the coldest lands.

Gemma Reguera is associate professor in the Department of Microbiology and Molecular Genetics, Michigan State University.

Manual of Clinical Microbiology, 10th Edition Blowout Sale

Limited Quantities so Act Fast

10TH EDITION
MANUAL OF CLINICAL MICROBIOLOGY
EDITORS JAMES VERSALOVIC • KAREN C. CARROLL
GUIDO FUNKE • JAMES H. JORGENSEN
MARIE LOUISE LANDRY • DAVID W. WARNOCK

Volume 1

Volume 2

Member Price: $215.95 NOW $139
Non-member Price: $269.95 NOW $199

Don’t miss this opportunity to own the most current edition of the *Manual of Clinical Microbiology*, now being sold at the lowest price ever! Once it’s sold out this edition will be gone!

Visit ASMscience.org to buy a copy of the print book, eBook, or by the e-chapter.

MCM10 sets the standard for state-of-the-science laboratory practice as the most authoritative reference in the field. This 10th edition represents the collaborative efforts of 22 editors and more than 260 authors from around the world, all experienced researchers and practitioners in medical and diagnostic microbiology.

2011. Hardcover in two volumes, 2,630 pages, color illustrations, indexes.

*When you buy books directly from ASM Press, you support the society that supports the science of microbiology.*

WEB: www.ASMscience.org | PHONE: 800-546-2416 | E-MAIL: books@asmusa.org

©2014 American Society for Microbiology Press
Strengthen your collection with high-impact research.

Edited by working scientists, ASM Journals are highly cited and highly regarded. Select an online subscription to provide your researchers with top-tier content at a maximum value.

**HIGHLIGHTS:**

1. ASM Journals publish over 20% of all Microbiology articles, while accounting for over 33% of all Microbiology citations.*
2. ASM Journals are indexed by trusted services such as PubMed®, MEDLINE®, Web of Science®, and many others.
3. Convenient tools including COUNTER 4 reporting and authentication management.

*as reported in the 2014 Release of the Journal Citation Reports®

**ASM PUBLISHES:**

4. out of 5 of the highest-cited journals in Microbiology.

3. Journals in the Top 20 of Microbiology ranked by Impact Factor:

- **#1** AEM, Applied and Environmental Microbiology®
- **#2** JB, Journal of Bacteriology®
- **#3** JCM, Journal of Clinical Microbiology®
- **#5** Antimicrobial Agents and Chemotherapy®
- **#2** CMR, Clinical Microbiology Reviews®
- **#3** mnbr, Microbiology and Molecular Biology Reviews®
- **#12** mBio®

**KUDOS** Pilot program with Kudos

**ITAs** Mandarin translations of the Instructions to Authors

We encourage librarians to add our free, open-access titles, genomeA™ and mBio®, to your collections.

New features for online subscribers in the 2015 subscription year:

**To receive a quote** for an institutional online package, or for information on how to order individual titles, please contact our Institutional Subscription Office:

Phone: 1-800-633-4931 (US and Canada) 1-205-995-1567 (Rest of the World)
Fax: 1-205-995-1588; asmjournals@subscriptionoffice.com