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Tyrrell Conway and Paul S. Cohen

Anaerobes in mixed biofilms degrade polysaccharides, sharing locally prepared sugars with facultative anaerobes that also colonize the intestine.
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First, some gossip. During a meeting last year, I heard a story from the director of a middle-ranking medical microbiology laboratory in southern Europe. Staff there had been perplexed by the unexpected, unexplained detection of a coryneform bacterium during routine screening of swabs from a nearby hospital. On five separate occasions, the organism had appeared on MacConkey agar and other cultures, and was at first difficult to identify. Only after the apparent contaminant was sent for further investigation to a reference laboratory elsewhere was part of the mystery solved. There, 16S rRNA gene sequencing showed the organism to be *Brevibacterium otitidis*.

I say “part of the mystery” because definitive identification of the bacterium still left unanswered the question of its origin. Maddeningly, the organism had turned up on plates being used to investigate specimens from five different patients. Could contamination of the culture media or their constituents be to blame? Careful searches proved negative, as did screening of the staff. Was there an unapparent source on the benches or other parts of the lab? Diligent scrutiny again proved negative.

But then the problem suddenly disappeared—and this led to a likely explanation. Someone noted that the cluster of five cases came to an end immediately after a student intern, employed at the center during the summer holidays, went away. Oddly, as a temporary worker, he was the only person around the lab who had not been swabbed. But as he was now many miles away on another campus, he was not investigated. The truth seemed inescapable—though not, of course, proven.

Cases of this sort are probably quite rare. The last one about which I myself heard first-hand evidence concerned an elderly veterinarian in the north of England over 40 years ago. To his acute, indeed chronic, embarrassment, the source of a contaminating virus in the center where he worked proved to be his own pet cat. Even when the cat fell under suspicion and was screened, it took over a week for a clear verdict to emerge. As the contaminant was apparently an enterovirus, the animal’s feces were thoroughly scavenged, but with negative results. Eventually, the problem turned out to have been originated by an ocular infection—which was quite obvious even on casual examination but which the vet had not noticed because he was far more used to dealing with racehorses and farm livestock than with cats and dogs. Ironically, he also had rather poor eyesight. As I said, the incident was all very embarrassing.

Although unusual, such episodes do remind us that the caricature of white-coated sleuths, wholly insulated from the microbial domain they are studying, is indeed a caricature. Perhaps microbiology students should be reminded (as physics students are) that investigators can themselves inadvertently interfere with the phenomena they are investigating.

Now let me take you to a scenario that developed recently at the West of Scotland Specialist Virology Centre in Glasgow, just a few miles north of the laboratory where the hapless veterinarian worked. Members of the staff there were using real-time PCR methods to evaluate throat swab samples sent in by primary medical centers participating in an influenza surveillance system embracing health boards in several different parts of Scotland. To their surprise, the Glasgow team found low-level influenza positive signals in 14 of the throat samples. Some were positive for a single influenza A or influenza B virus, while tests on other specimens indicated that more than one virus was present.

At that time, both influenza A and influenza B activities were very low in Scotland, no detec-
tions having been reported through monitoring schemes either there or in other parts of the United Kingdom and Europe. The team concluded, therefore, that the weakly positive signals did not reflect actual infections but might instead be a result of contamination from recent influenza vaccinations. Indeed, immunization with live attenuated influenza vaccine (LAIV) had been conducted over recent months in Scotland, and had for the first time included 2- and 3-year old children.

As Susan Bennett and her colleagues report in the *Journal of Medical Microbiology* (64:466, 2015), 7 of the 14 samples suspected of containing LAIV—including one which, on the initial influenza screen, was weakly positive for influenza B only—did indeed prove positive in a H2N2-specific assay. The remaining seven samples were negative, possibly because they had LAIV contamination at levels below the cutoff point of the assay.

“Of the 12 patients with a vaccine history, three were given inactivated influenza A vaccine (IAV) and the remaining nine had not received any influenza vaccination,” the authors write. “The two patients without a vaccine history were 29- and 75-year olds and therefore would not have been offered LAIV. Since none of the individuals were given LAIV, we cannot rule out the positives being a result of vaccine replication/shedding post vaccination. The false-positive results may be due to transmission of LAIV from recent vaccinated individuals as previous studies have shown that live vaccine can be, albeit rarely, transmitted between those given vaccine and close contacts.”

Could it be that intranasal administration of LAIV might allow large quantities of influenza A and influenza B viruses to become aerosolized, thereby contaminating the local environment? This might mean that patients with respiratory infections, entering that environment, would be unwittingly infected by the live, attenuated viruses. In an attempt to settle the question, Bennett and her coworkers got in touch with each of the primary health centers that had been conducting LAIV immunizations on site. All of the centers said it was indeed likely that both vaccinations and sampling had occurred in the same room. This finding mirrored the outcome of a previous investigation elsewhere which revealed the detection of IAV in environmental samples from areas where immunization clinics had taken place, and that vaccine could be recovered from surfaces for up to 66 days afterwards (T. Curran et al., J. Med. Microbiol. 61:332, 2012).

While reporting their findings, the Glasgow virologists issue a warning that laboratories using highly sensitive real-time PCR techniques should be conscious of the risk of LAIV contamination occurring within clinical samples taken at times when vaccination programs are running. Moreover, they say, public health bodies ought to be aware of this problem when they interpret surveillance data.

“Laboratories should consider using a LAIV-specific assay to confirm weak influenza A or B positive results, especially those detected concurrently with vaccination,” they write. “Using such a test will ensure that this type of contamination will not affect public health surveillance data or patient management and will prevent unnecessary laboratory expenses should such results be mistaken for PCR contamination.” They even find it necessary to warn centers that offer flu immunization to take particular care to decontaminate their environment before sampling patients with respiratory illnesses.

As with my story about *Brevibacterium otitidis*, it’s worth emphasising that the Glasgow incident did not occur in a temporary medical center in Nepal, or a hard-pressed field hospital in Ethiopia. Nor was it something that took place in a laboratory where early virologists were learning their craft, and taking substantial but unknown risks, in the first half of the 20th century. The incident happened in the present day, in a well-established, well-equipped facility staffed by highly trained, highly qualified, highly responsible microbiologists. Vigilance can never be set aside.
Current Topics

RESEARCH ADVANCES

Wind-Blown Microbes Add only Modestly to Life in Greenland Glaciers

Barry E. DiGregorio

Aeolian, or wind-blown, microorganisms pelt the Greenland Ice Sheet all winter long but remain inactive until some of that ice melts to form metabolically active pools during the short summer growth season, according to Michaela Musilova from the University of Bristol in the United Kingdom and her collaborators from there and the Universities of Edinburgh and Leeds as well as the Woods Hole Oceanographic Institution in Woods Hole, Mass. Even then, however, those inputs to microbial communities that form in melt holes along these glaciers are “limited,” with the communities themselves being “stable and active” throughout the summer, “making them important contributors to biogeochemical nutrient cycling on glaciers,” the researchers note. Details appeared 20 March 2015 in Frontiers in Microbiology (6:193; doi:10.3389/fmicb.2015.00193).

During the summer, cylindrical holes in glaciers fill with water and debris, called cryoconite, forming small pools in which microbial communities can flourish. Stable communities within holes on the glacier contain mainly proteobacteria, cyanobacteria, and actinobacteria, according to Musilova and her collaborators. An open question was whether aeolian sources augment these established microbial mixtures, they note. “However, the dominant bacterial taxa in the aeolian samples—Firmicutes—did not establish themselves in local glacier surface communities.” As for the dust itself, it originates almost entirely from deserts and dry areas in eastern Asia, such as the Gobi desert.

“The evolution of the microbial community within cryoconite holes is very interesting,” says Andrew Fountain at Portland State University in Portland, Ore., who studies similar communities in Antarctica. “Seeded initially with aeolian biota in snow, then within a few weeks only a few were left, indicating a natural filtering of those who thrive in such a habitat. The fact that the microbial communities remained stable over time, once established, is useful to know for future investigations, saving time and energy sampling multiple holes over the season.”

These results raise questions as to how important it is for those established microbial communities to survive winters and then establish active communities during early spring melts, Fountain continues. A related question is how important input microbiota are for maintaining community structures in terms of adding different species versus bringing in new sources of nutrients, he says. “Perhaps indirect measures of water chemistry, such as electrical conductivity and partial pressure of gases in cryoconite waters, may be used as an index of microbial activity once the microbial community becomes established for the season.”

Little is yet known about the genes that collectively operate within these glacier surface microbial communities, according to Musilova, who plans metagenomics analyses to address such questions. “It would also be very interesting to learn more about the aeolian microbes to determine their origin, whether they are viable upon deposition on the glacier surface, and whether they are indeed out-competed by the local communities,” she says. “Here we determined the isotopic signatures of the organic carbon in the samples, but...
Host Signals Can Trigger Virulence in Candida albicans

Shannon Weiman

The virulence of Candida albicans changes in response to signals within and from the host, according to several researchers who spoke during the 28th Fungal Genetics Conference, held in Pacific Grove, Calif., last March. In some cases those host signals render this yeast innocuous. In other circumstances, however, C. albicans upregulates virulence factors in response to host signals, becoming an outright pathogen. A recent thrust of research is to reveal those signals that trigger such responses as well as the fungal regulatory pathways that respond to them, with a longer-term goal of combating infections not by killing this yeast, but by blocking virulence.

“C. albicans is the dominant [fungal] commensal of the human gastrointestinal tract, as well as the most common invasive fungal pathogen,” says Suzanne Noble of the University of California, San Francisco. In its commensal state, gastrointestinal induce transition (GUT) cells of C. albicans are attenuated for mutualism. However, in its pathogenic invasive “white” form, metabolic and other adaptive changes enable these changed yeast cells to invade the host. This switch of C. albicans is triggered by gut environmental signals and mediated by the Wor1 transcription factor, she says. “The resulting GUT cells differ morphologically and functionally from previously defined cell types, and express a transcriptome that is optimized for the digestive tract ... exhibiting a striking reorientation of cellular metabolism towards nutrients available in the distal mammalian GI tract.”

Carbon dioxide and N-acetylglucosamine are candidate signals from the host controlling this Wor1-induced transition, Noble continues. “Our results indicate that the ability of a commensal organism to produce disease is not merely a consequence of impaired host immunity. The identification of specialized states for C. albicans commensalism and virulence offers opportunities for prevention as well as treatment of clinical diseases produced by this important human pathogen.”

Meanwhile, temperature changes unambiguously can increase the virulence of C. albicans, according to Michelle Leach of the University of Aberdeen in the United Kingdom. When exposed to relatively higher tempera-
MINITOPIC

Vaccine Update

Recent developments involving vaccines include:

- The Pan American Health Organization/World Health Organization in April declared the Americas the first region in the world to be free of endemic transmission of rubella—culminating a 15-year effort to deploy the measles-mumps-rubella vaccine throughout the 45 countries and territories of North, Central, and South America.
- Use of microneedle patches could make it easier to deliver the measles as well as other vaccines, according to official at the Centers for Disease Control and Prevention (CDC) in Atlanta, Ga., and their collaborators at nearby Georgia Institute of Technology.
- Because measles infections can suppress immune responses for as long as 3 years, the measles vaccine “protects polymicrobial herd immunity” longer than previously believed, helping to explain the long-term benefits of this vaccine, according to Michael Mina of Princeton University in Princeton, N.J., and the School of Medicine at Emory University in Atlanta, and his collaborators at nearby Georgia Institute of Technology.
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This takeover can be followed in vitro by growing these two bacterial species together on a plastic substrate or on airway epithelial cells from individuals with CF, according to O’Toole. Identifying and determining the mechanisms underlying the takeover “are the initial steps to understanding how CF microbial lung communities develop and affect patient health,” he says.

When *P. aeruginosa* steals iron from other bacteria, levels of the molecule it makes called *Pseudomonas* quinolone signal (PQS) also rise, according to Oglesby-Sherrouse’s graduate student Angela Nguyen. During coculture in vitro by growing these two bacterial species together on a plastic substrate or on airway epithelial cells from individuals with CF, according to O’Toole. Identifying and determining the mechanisms underlying the takeover “are the initial steps to understanding how CF microbial lung communities develop and affect patient health,” he says.

NEW IN ASM JOURNALS

In Cystic Fibrosis, Iron-Grabbing *Pseudomonas* Outcompetes *S. aureus*

David C. Holzman

Of the two major bacterial species that colonize the lungs of cystic fibrosis (CF) patients, *Pseudomonas aeruginosa* eventually dominates *Staphylococcus aureus*. The faster and more fully the former overshadows the latter, the more likely is the host to experience morbidity and mortality, according to George A. O’Toole of the Geisel School of Medicine at Dartmouth University in Hanover, N.H., and his collaborators. That takeover depends in part on the ability of *P. aeruginosa* to deprive *S. aureus* of much-needed iron, according to Amanda Oglesby-Sherrouse at the University of Maryland, Baltimore, and her collaborators. Details appeared in separate reports 29 April 2015 in the *Journal of Bacteriology* (doi:10.1128/JB.00059–15 and doi:10.1128/JB.00072–15, respectively), along with a commentary in that same issue (doi:10.1128/JB.00303–15).

*P. aeruginosa* drives *S. aureus* into anaerobic metabolism, slowing the latter’s growth while appropriating lactate and iron to accelerate its own growth, according to O’Toole. This process depends on the quorum sensing molecule 4-hydroxy-2-heptylquinolone-N-oxide (HQNO) and two iron-binding molecules, pyoverdine and pyochelin, boosting expression of fermentation pathway genes in *S. aureus*, he says.

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When *P. aeruginosa* steals iron from other bacteria, levels of the molecule it makes called *Pseudomonas* quinolone signal (PQS) also rise, according to Oglesby-Sherrouse’s graduate student Angela Nguyen. During coculture in iron-poor environments, increases in PQS levels plus increases in levels of various 2-alkyl-4(1H)-quinolones...
(AQs), including HQNO, enable \textit{P. aeruginosa} to outcompete other bacterial species in its environment, particularly \textit{S. aureus}, according to Oglesby-Sherrouse. “Both bacterial species must be cultured together for this phenomenon to occur, as you can’t just provide culture supernatant from \textit{P. aeruginosa} to block growth of \textit{S. aureus},” she says. “This indicates that \textit{P. aeruginosa} must sense \textit{S. aureus}.” Previously, she notes, most of what is known about iron regulation in bacterial species was learned from studies of bacteria grown in pure cultures.

“These two papers . . . give some of the first evidence regarding how host micronutrients [affect] interactions of two prevalent CF pathogens and provide insights into the mechanism controlling \textit{P. aeruginosa}-mediated succession in the CF lung,” note Patricia M. Barnabie and Marvin Whiteley of the University of Texas, Austin, in the commentary that accompanies the two reports. “The control of such host-modulated bacterial interactions is thus a largely unexplored field that may one day prove a fertile ground for medical innovation.”

\textit{Fungi are unappreciated, but remarkable properties and applications of fungi in environmental biotechnology.” Moreover, \textit{Penidiella’s} geochemical properties are relevant to recovering this element, bioremediating sites where it is a contaminant, and for producing novel inorganic biomaterials.}

Dysprosium is considered the single most critical element for some kinds of clean energy production technology, according to officials of the U.S. Department of Energy. Most dysprosium is mined in China, and prices soared...
The rare-earth element dysprosium contaminates industrial and mining sites. Researchers were able to isolate a fungal species, *Penidiella* sp. T9, that takes up and concentrates dysprosium and other rare-earth elements from such sites. (Image © slovegrove/iStockphoto.)

from $7 per pound in 2003 to $130 per pound in 2010. A low-cost process for recovering dysprosium from mining sites and contaminated water at industrial sites could increase its supply and reduce costs. With current concerns over sustaining the supply of dysprosium and other valuable rare-earth elements required for clean energy technology, “fungal systems have a clear potential for the immobilization and biorecovery of these important substances,” Gadd says.

There are precedents for using microorganisms on an industrial scale to recover metals and radioactive elements, according to Gadd. Sulfate-reducing bacteria are used to precipitate and recover metals such as zinc in a commercial system available through Paques BV, headquartered in the Netherlands. Other industrial-scale systems rely on acidophilic bacteria to make sulfuric acid for leaching copper, gold, and uranium, he says. Amid interest in finding microbes to recycle rare-earth elements, he adds, “so far there are no industrial processes.”

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

**RESEARCH ADVANCES**

**Outbreak Spurred Several Approaches to Developing Ebola Therapeutic Products**

Jeffrey L. Fox

Several distinct types of Ebola therapeutic products are under development—some more or less conventional antiviral agents, while others depend on more recently developed techniques to disrupt viral gene activity or use antibodies to bind the virus and trigger host immune responses.

Some of these candidate drugs block replication of the virus genome. For example, BCX4430, under development by BioCryst of Research Triangle Park, N.C., targets the RNA polymerase of the Ebola and Marburg viruses. It proves effective in treating nonhuman primates infected with either of those viruses. Favipiravir, also called T-705, initially was developed by Toyama Chemical, part of FujiFilm Corp. of Tokyo, Japan, because of its activity against influenza virus strains. Although the drug failed to help patients with advanced Ebola infections during the recent outbreak in West Africa, it appeared to help in preventing deaths among those with more moderate infections.

Sequence-specific inhibitors of the Ebola virus include phosphorodiamidate morpholino oligomers (PMOs), being developed by Sarepta Therapeutics of Cambridge, Mass., and lipid-encapsulated siRNA oligomers, being developed by Tekmira Pharmaceuticals of Vancouver, British Columbia, Canada. The Sarepta PMOs target Ebola or Marburg virus matrix proteins. “These compounds have protected up to 80–100% of nonhuman primates to Ebola and Marburg virus challenge infections, respectively,” says Michael Wong of Sarepta. Despite such promising findings, however, the Department of Defense (DoD) Joint Product Management Office of BioDefense Therapeutics recently dropped its sponsorship of PMOs.

DoD continues to support the RNA silencing candidate products that Tekmira is developing, including its lead candidate, TKM-Ebola-Guinea, which was adjusted to accommodate minor sequence changes in the genome of the viral strain that circulated in West Africa during the outbreak. The lipid-encapsulated siRNA “triggers” target the genes VP35 and L polymerase of the Ebola virus, which function in viral replication and, in the case of VP35, also in blocking the host immune response to the virus, according to Tekmira.

Meanwhile, several mixtures containing specific monoclonal antibodies (mAbs)—typically, two or more mAbs aimed at more than one target on the Ebola virus—are now being evaluated as therapeutic products. Treatment results are anecdotal, but similar mixtures of these mAbs seem to be having an effect in patients infected with the Ebola virus, according to Gary Kobinger of the National Microbiology Laboratory in Winnipeg, Canada, part of the Public Health Agency of Canada (PHAC).

Prominent among these candidates
is ZMapp, a triple monoclonal cocktail of humanized antibodies that are directed against the Zaire species of Ebola virus. Originally developed several years ago in collaboration with the U.S. Army Medical Research Institute of Infectious Disease (USAMRIID) in Frederick, Md., commercial development of ZMapp was handed over to Mapp Biopharmaceuticals in partnership with LeafBio, both in San Diego, Calif. ZMapp is produced in tobacco plants at Kentucky BioProcessing in Owensboro. A similar mAbs cocktail was formulated by PHAC researchers in Winnipeg. Efforts are under way to produce enough of these and similar mAbs to evaluate them in clinical trials.

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

NEW IN ASM JOURNALS

**Fungus Gardening Ant Gut Flora Are Few but Intriguing**

Fungus gardening termites have diverse gut microbiota. Now, for the first time, researchers from the University of Copenhagen have investigated that of fungus-gardening ants, whose gardening techniques are fundamentally different. Termites predigest their plant substrate whereas ants deposit it directly on the fungus garden. Panagiotis Sapountzis et al. show that there are but four major species of gut bacteria in *Acromyrmex* leaf-cutting ants, but that these are intriguing. A *Rhizobiales* symbiont inhabits the hindgut as a biofilm, and fixes atmospheric nitrogen—just like congeners that abide in root nodules of legumes on human farms. Obtaining sufficient nitrogen “is an obvious challenge when the farmers no longer hunt the meat of insect prey,” says Sapountzis. He notes that “leaf cutting ants are serious pests for human agriculture as they defoliate many crops, to nourish their underground fungus farms.” The research might lead to environmentally friendly biopesticides to replace the contaminating organochlorines now used, he says.

CURRENT TOPICS

NEW IN ASM JOURNALS

Cervical Cancer mRNA Test Offers More Specificity than Gold Standard Test

Around 4,600 cases of cervical cancer are diagnosed and 1,500 women die annually in Germany (comparable numbers in the U.S. are nearly 13,000 and 4,100). Thomas Iftner of Tubingen, Germany, et al. compared two tests for detection of high-risk human papilloma virus, one that detects mRNA of the viral oncogenes, and the other—the current gold standard test—that detects viral genome DNA.

“In cervical cancer screening, healthy individuals are screened for precursor lesions of cervical cancer and/or for the presence of the major risk factor—infection with high risk HPV types,” says Iftner. “The RNA-based assay is more specific, and as sensitive for detection of high grade precancerous lesions as the DNA-based test.” That higher specificity means the test could reduce false positives, and thus overtreatment in cervical cancer screening, he explains.


NEW IN ASM JOURNALS

Electrical Currents Show Promise for Mitigating Biofilms

Amperage as low as 5 μA over 7 days, intermittent current as little as four hours/day, and 23 of 24 different disc and electrode combinations reduced biofilms of Staphylococcus aureus, Staphylococcus epidermidis, and Pseudomonas aeruginosa biofilms, according to Robin Patel et al. of the Mayo Clinic, Rochester, Minn0. Electrical current reduced biofilms of 25 of 33 strains tested, representing 13 species.

“This study shows potential use for electricity in treating biofilm infections, specifically those associated with implanted materials,” says Patel. If ultimately successful, this strategy could minimize unintended consequences of antibiotic use, such as resistance.”


NEW IN ASM JOURNALS

Band-Aid Vaccine Cures Chronic Ear Infections in Animal Model

No vaccine exists to prevent or treat the estimated 66–330 million cases of chronic otitis media that occur annually worldwide. Now Lauren Bakaletz of Nationwide Children’s Hospital, Columbus, Ohio, et al. have developed a therapeutic vaccine that cured non-typeable Haemophilus influenzae (NTHI), the most common culprit in chronic ear infections. “The work shows that therapeutic immunization using a Band-Aid placed behind the ear eradicated NTHI from the middle ear in an animal model,” says Bakaletz.

“The vaccine was picked up by dendritic cells, which then induced a protective response via cytokines and specific antibodies that were present in the middle ears of immunized animals. This study is the first to demonstrate therapeutic immunization with a Band-Aid vaccine to resolve experimental ear infections caused by NTHI.”


NEW IN ASM JOURNALS

Wild and Domestic Birds and Escherichia: the Human Risk Evaluated

Domestic backyard poultry and wild birds are potential sources of human infection. Blyton and Michaela Blyton of Australian National University show that Escherichia strains isolated from backyard domestic poultry were genetically distinct from isolates from wild birds in wildlife rehabilitation clinics, wilderness, and suburbia. Escherichia from backyard poultry and from wild birds were more likely to carry virulence-associated genes, but no differences were found between the Escherichia communities in suburban and wild birds. The results suggest that there is little human-to-bird transmission in Australian urban environments, but that the higher level of antimicrobial resistance in backyard poultry and birds at rehabilitation centers could pose a risk to human health.

“This highlights the need for more rigorous hygiene practices when caring for orphaned, injured, and sick wildlife,” says Blyton. Antimicrobials, she says, should be administered only where infection is strongly suspected or confirmed, and avoided when survival is unlikely.

NEW IN ASM JOURNALS
Copper Surfaces Destroy Human Norovirus

Lee-Ann Jaykus of North Carolina State University and doctoral student Clyde Manuel report the first study to show that copper kills human norovirus. They deposited human fecal samples containing either infectious virus or virus-like particles onto five different copper alloys, or stainless steel, as a control, and tested virus survival. The copper alloy surfaces all “virtually abolished” the receptor-binding ability of HNV within 10 minutes, while the virus was “very stable” on stainless steel.


NEW IN ASM JOURNALS
New Evidence Silver Resistance Is Emerging

Due to broad-spectrum antimicrobial properties, silver has become a popular additive for medical treatments and devices, especially for burn and wound care. Now Phillip J. Finley of Mercy Hospital, Springfield, Mo., et al. provide evidence of emerging silver resistance. They found Klebsiella pneumoniae and Enterobacter cloacae to be highly resistant to many commercial silver-based wound dressings, and scanning electron microscopy observations suggested that bacteria might be neutralizing silver once it is bound to the cell membrane, says Patel. “The development of acute silver resistance would have extensive consequences on current wound therapies and patient care.”

ANTIBODIES
FOR INFECTIOUS DISEASES

Editors: James E. Crowe, Jr., Professor, Pediatrics, Pathology, Microbiology and Immunology, Vanderbilt University, and Director, Vanderbilt Vaccine Center; Diana Boraschi, Research Director, National Research Council; Rino Rappuoli, Global Head of Vaccines Research, Novartis Vaccines and Diagnostics

Provides a broad survey across important aspects for understanding mechanisms of immunity to infectious disease, including general features of antibodies and approaches to antibody discovery, individual chapters highlighting antibodies against particular pathogens, and state-of-the-art technical advances and expression systems.

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Improving How We Communicate about Infectious Disease Risks

Several strategies are available to engage diverse stakeholders, correcting misperceptions and empowering them to act

Robert S. Littlefield

Although preventing and controlling infectious diseases have raised life expectancy rates, public awareness about these diseases and how they can be controlled has declined. Inaccurate information, misperceptions, and questionable practices thus threaten to reverse gains made in public health.

These threats fall across a broad spectrum. For example, inaccurate information about the hazards of immunization has led to decreased vaccination rates, increased consumption of raw milk has led to a rising number of illnesses and deaths linked to dairy products, misuse of antimicrobial agents has resulted in the emergence of multiple antibiotic-resistant organisms, and the widening use of antiseptic products such as triclosan is raising environmental concerns. In facing these threats, microbiologists and policy makers need to develop communication strategies to engage the public when dealing with these issues. These strategies define key terms and then build a framework for introducing communication strategies into public discussions surrounding infection disease control and prevention.

Communications Strategies Start with Key Definitions

Key terms need defining, beginning with risk and crisis communication. Risk and crisis communication can be defined as “an interactive process, exchange of information and opinion among individuals, groups, and institutions . . . revealing various threats or risks to the community,” according to Michael J. Palenchar of the University of Tennessee, Knoxville, and his colleagues. Risk communication involves multiple entities, whose diverse members disagree about the nature and severity of particular risks.

Risk may be considered the sum of perceived hazards plus the level of outrage felt by those most directly involved, according to consultant Peter Sandman of Brooklyn, N.Y. If the hazard and outrage are high, the perception of risk is great. If either the severity of the hazard or the level of outrage is low, the perception of risk will vary. In either case, without consensus, decision-makers face difficulties gaining compliance with their instructional risk communication.

To be successful with multiple publics, agencies and organizations cannot merely ignore or minimize the concerns of those who disagree or question their views. Merely dismissing them as “the crazies” because their arguments seem illogical or appear to manipulate the facts is ineffective. Rather, organizations need to keep the lines of communication open with their stakeholders and publics, even when the agencies do not value the views that are being introduced.

A second term to define is vulnerable publics.

SUMMARY

➤ Several strategies help when reaching out to different stakeholders, some of whom are difficult to communicate with, to correct misperceptions and empower them to act when facing infectious diseases.

➤ These strategies define key terms and build a framework for holding more productive public discussions surrounding infection disease control and prevention.

➤ Risk may be considered the sum of perceived hazards plus the level of outrage felt by those most directly involved in dealing with a particular risk, particularly stakeholders and vulnerable publics.

➤ Decision-makers need to realize that vulnerable publics respond differently to risks than do other groups.

➤ Three broad strategies are available to engage different publics and to help them respond better to risks.
With those definitions in mind, it is appropriate to consider some of the challenges facing organizational decision-makers regarding how to develop and present risk and crisis messages. Two key challenges include the need for decision-makers to shift to an audience-oriented focus with knowledge of cultural variables, and that vulnerable publics respond differently to risks than do other groups.

Dealing with the first challenge requires organizations to develop a more nuanced understanding and application of cultural variables. Early risk and crisis scholars focused on strategies an organization could use to improve or restore its image with stakeholders or publics affected by the crisis situation. However, this sender-focused analysis reflected the perspective of the organization and failed to take into consideration the perceptions of the receivers of the risk messages.

Larry Sarbaugh of Michigan State University sought to change this approach by identifying four variables to manage when adopting an audience-oriented focus. They include use of code systems, perceptions about relationships and intent, knowledge and acceptance of normative beliefs and values, and worldviews. A code system refers to the words used when discussing normative beliefs and values, and worldviews. A code system includes acronyms that are unfamiliar to the audience, the message will not be well received.

When vulnerable publics question the intent of an organization, they are unlikely to comply with the directives that it issues. For example, if an organization has a bad history with a particular individual or group, there is less likelihood that group members will perceive the intent of the organization as helpful. Rather, the individuals in the group may suspect the organization of having an ulterior motive to harm them.

A third variable involves learning about and accepting normative beliefs and values of the affected publics. Vulnerable publics and organizations have different belief systems. For example, if a vulnerable public subscribes to a magical or spiritual approach to health, claiming that vaccinations allow infectious illnesses to be inflicted upon healthy bodies, members of this public group are likely to reject standard treatments because of the belief that those treatments will cause harm to whoever is vaccinated. Organizations thus need to consider the beliefs and values of those who will receive the message and consider how they can account for those conflicting perspectives when presenting their messages.

Finally, conflicting worldviews challenge organizations seeking to instruct their stakeholders and publics about how they should behave or
respond to risks. Essential elements of worldview include nature of life, purpose of life, and relationship of humans to the cosmos. If members of a vulnerable public believe that the nature of life is hardship, the purpose of life is to prepare for the afterlife, and their relationship to the cosmos is one of subordination, they may be unlikely to believe that vaccines or medical treatments will be useful or even appropriate. Because they are prepared to accept their fates, taking steps to mitigate or reduce the risk of the disease being faced would be considered a wasted effort. Thus, designing and presenting messages to this group requires careful consideration.

The challenge of adopting an audience-centered focus contributes to the second issue facing risk and crisis communicators. In being vulnerable, publics respond differently to risks. For example, how do they compare the risk of food poisoning from consuming contaminated hamburgers to that of becoming infected by the Ebola virus? The risk of illness and death from consuming raw milk may not stop individuals from using such dairy products. Moreover, the perceived risk from being vaccinated may be considered greater than the perceived risk of contracting an infectious disease.

Depending upon one’s ethnicity, country of origin, economic status, education level, access to information, literacy, and social standing, the receiver of a message may be more or less likely to respond according to cultural beliefs and values than directly to the content of the message. The variability in responses from different publics receiving risk messages complicates message making for organizations seeking to account for differing attitudes towards those risks. The belief that one message can have the same effect on all receivers is flawed. Ideally, these three elements will be balanced to create a message that audiences receive well, according to Sellnow and her colleagues.

Strategies to Improve Compliance

Three strategies are available to engage different publics and to help them respond better to risks. These strategies involve using elements from the IDEA Model of Instructional Risk Communication, applying decision-making tensions, and becoming more mindful of socio-cultural variables that affect compliance.

Instructional risk communication specialists Deanna Sellnow and Timothy Sellnow at the University of Kentucky introduced the IDEA Model of Instructional Risk Communication. According to that model, three key elements affect message compliance: internalization, explanation, and action. First, messages need to be designed to connect with their intended audiences, capturing their attention and enabling them to internalize the contents of each message. To be appropriately explanatory, messages must provide details of what is happening, why, and what is being done to mitigate or remove risks. Finally, messages need to provide options for action because people want to know what to do to protect themselves and their families or communities against the risks they face.

The second strategy, which my collaborators and I developed, takes into account decision-making tensions faced by organizations creating and disseminating risk messages to the public. We identified seven tensions that decision-makers face when responding to risks: 1) timeliness of the information, 2) amount of information, 3) certainty about the information being released, 4) control of the organization’s narrative about the risk, 5) level of responsibility for the crisis, 6) prioritization of interest, and 7) emotional connection with the publics (see table). For each tension, extremes exist. For example, for the tension of timeliness, organizations must decide whether to release the information immediately or how long to delay its release.

In determining what levels of tension must be accounted for when creating and disseminating messages, organizations taking a culture-centered approach can expect to benefit

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<td>Timeliness</td>
<td>Immediate - Delayed</td>
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<td>Amount of Information</td>
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<td>Certainty</td>
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the most. For example, including stakeholder groups and members of different publics on the risk communication team within an organization will provide insights about how to account for tensions and respond most effectively to different target groups.

A third strategy involves being mindful of socio-cultural variables that affect how messages are received. For example, in following this strategy, my team, including Kimberly Cowden and Will Hueston, developed practical strategies for engaging New Americans, immigrants, and Native Americans when developing risk messages. Thus, we included members of different multicultural community groups on our team when we developed messages about specific risks, and then we asked cultural agents, who are trusted by their communities, to present that information.

We found it valuable to take time to build relationships with different cultural communities to increase credibility and trust, to explore different ways to listen to and involve publics in discussions about risk and crisis situations, to understand that cultural views about health crises vary, to take religion and cultural practices into account when selecting spokespeople, creating the message, and disseminating it to stakeholders and the public; to recognize that individual learning styles vary and what one group may find preferable, another might not value; to understand that the literacy levels of the publics will affect their ability to follow written directions; and to account for whether affected groups have had an adversarial or hostile relationship with organizations, including government agencies, because it may affect their feelings and how willing they will be to accept risk instructional communication.

Following these three strategies can improve the likelihood that those receiving instructional risk communications will respond as directed. Focusing on internalization, explanation, and action helps organizations pay closer attention to how risks are affecting particular members of the public and how best to prevent or mitigate a crisis. Identifying the tensions and using members of the affected publics to gain insight on finding the balance between these tensions while a crisis is in play will help organizations to navigate situations in the best interests of all who are involved. Finally, being attentive to cultural variables will enable decision-makers to build a fuller understanding of different factors that affect receptivity among vulnerable publics.

Conclusion

Vulnerable publics having differing cultural perspectives continue to question health professionals about the safety of vaccines, forcing health officials to tailor risk and crisis messages that better provide relevance, meaning, and application of those messages to skeptics. If health professionals engage stakeholders and publics in ways that address their concerns and misperceptions about infectious disease prevention and control strategies, the scientific community will be more able to control these diseases in the future.

Robert S. Littlefield is Professor of Communication, North Dakota State University, Fargo.

Suggested Reading


Archaellum Moves Archaea with Distinction

The archaellum is a rotating motility appendage found only in the archaea; it assembles with a type IV pili-like mechanism

Sonja-Verena Albers and Ken F. Jarrell

Cell motility in pure cultures of halophilic archaea was first observed almost a century ago and, in methanogens, at least as long ago as 1951, when *Methanococcus vannielii* was first isolated. Although *M. vannielii* was reported as being motile, no flagella were observed until nearly three decades later. Other researchers from that era reported seeing fimbriae (pili) or flagella on a variety of different archaea, including methanogens, halophiles, and *Sulfolobus*. However, during that period the members of the archaea were still considered bacteria, predating the groundbreaking realignment of the tree of life into the three domains of Archaea, Bacteria, and Eukarya by Carl Woese.

Dieter Oesterhelt at the Max Planck Institute for Biochemistry in Martinsried, Germany, began studying the swimming behavior of the haloarchaeon species *Halobacterium salinarum* during the 1980s, soon after Archaea was recognized as a third domain of life. With Maqsudul Alam, who is now at the University of Hawaii, Honolulu, they showed that the structure responsible for the motility of this microorganism differs from the well-studied bacterial flagellum, even though both structures had some properties in common. Oesterhelt and Alam found that the flagella of *Ht. salinarum* form an unusual, right-handed helix rather than the almost universal, left-handed helix of bacterial flagella. In addition, unlike almost all bacterial flagella known at the time, the protein composition of the halophile’s flagella appeared to be more complex, showing up as three bands of intensities on gels, each with a ladder-like series of minor bands, suggesting considerable variations in composition.

When the flagella of such cells are tethered, the cells switch between rotating either in the clockwise (CW) or counter-clockwise (CCW) direction, with CW rotation resulting in the pushing of the cells while CCW rotation results in pulling. During this switch in rotation directions, the flagella bundle does not fly apart, as happens with bacterial flagella. These early observations suggested that the archaecal motility structure differs from the bacterial flagellum, even though superficially both structures appeared to share features.

Evidence Builds for Archaea Having a Distinct Structure for Motility

Support for archaea having a different motility structure came in 1988 from Martin Gerl and Manfred Sumper at the Universität Regensburg in Regensburg, Germany. From *H. salinarum*, they uncovered five genes associated with the motility apparatus of this microorganism—encoding proteins whose amino acid sequences are highly conserved. However, although they were looking for flagellin proteins, these proteins share no significant sequence similarity with bacterial flagellin proteins or with any other proteins in the protein databases at that time. In other words, they encode for a motility structure that is unique to Archaea.

**SUMMARY**

➤ The proteins encoding the archaellum, the locomotive structure of archaecal species, are distinct from bacterial flagellar proteins but resemble the bacterial type IV pilus proteins.

➤ Archaellum structural proteins (archaellins) carry signal peptides and most are N-glycosylated, other features that set them apart from bacterial flagellin proteins.

➤ Although the bacterial type IV pilus can “twitch,” the archaecal motility structure rotates. Further, ATP drives the rotation of the archaellum, whereas the proton motive force drives the bacteria flagellum.

➤ Flal, the only ATPase in the archaellum operon, is bifunctional—essential not only for the assembly of the archaellum filament but also for the completed organelle to rotate.

➤ While the major function of the archaellum is motility, it can also help archaecal cells attach to various surfaces or to interact with other cells.
Moreover, these archaeal “flagellin” proteins possess another unusual feature, setting them apart from those made by bacteria. Specifically, they are glycoproteins with an attached N-linked glycan, which is added to the protein along the external face of the cytoplasmic membrane of the archaeal cell.

These features led Gerl and Sumper to surmise that the process for assembling this archaeal motility structure differs from that used in making the bacterial flagellum. Archaeal flagellar structural proteins from many other archaea are also N-glycosylated, making this modification widespread. Interfering with N-glycosylation of these proteins dramatically affects flagella assembly and function in those archaea that have been studied. Meanwhile, although some bacterial flagellin proteins are also glycoproteins, so far they all seem to possess O-linked rather than N-linked glycans.

**Genomic Sequencing Confirms Differences between Archaea and Bacteria**

The first archaeal genomic sequence—that for *Methanocaldococcus jannaschii*—was determined in 1996. For it and for all the archaeal genomes that followed, no genes that were homologous to ones encoding proteins important for the biosynthesis of the bacterial flagellum could be identified, even in organisms that had flagella and, in many cases, possessed obvious homologues of bacterial genes needed for chemotaxis.

Yet another difference stands out between the archaeal structure and the bacterial flagellum. As first reported by Martin Kalmokoff in the Jarrell laboratory of Queen’s University in Kingston, Ontario, Canada, the archaeal flagellin proteins include signal peptides, unlike bacterial flagellin proteins, which are not made as preproteins.

Subsequently, it was realized by Dave Faguy of the Jarrell group that the filament proteins of the archaeal motility structure include both a signal peptide and a conserved N-terminal region that shares high sequence similarity to that found in bacterial type IV pilins. With increasing numbers of archaeal genomes sequenced and with other genetic work on the halobacterial and methanococcal motility structures, researchers realized that the operon encoding the archaeal structures is not related to the bacterial flagellum, but rather to the bacterial type IV pilus and the first model of archaella assembly incorporating these ideas appeared.

As in type IV pilis, the N-terminal signal peptide of the filament protein is processed by a dedicated type IV peptidase that is homologous to the bacterial prepilin peptidase (PilD).
In archaea, this enzyme was first identified in methanogens by Sonia Bardy of the Jarrell group and called FlaK (where it may only process archaellin proteins) and subsequently in *Sulfolobus* by Zalan Szabo of the Albers group, who named the enzyme PilD (where this enzyme processes all type IV pilin-like proteins). Like PilD, both FlaK and PilD possess conserved aspartic acid residues that are critical for enzyme activity.

These similarities to type IV pili suggested that the structure of the archaeal filament is similar to type IV pili assembled in a three start helical way, an idea that was confirmed by Shlomo Trachtenberg at Hebrew University, Hadassah Medical School in Jerusalem, Israel, and colleagues. Critically, that structural work indicated that the archaeal appendage lacks the central channel essential for passage of subunits in the assembly of the bacterial flagellum, where new subunits are added at the distal end of the growing structure. Moreover, the archaeal fla operon encodes an ATPase (FlaI) and an integral membrane protein (FlaJ) that are homologous to the pilus assembly ATPase PilB and the platform protein PilC that forms the assembly platform of the type IV pilus in the inner membrane of gram-negative bacteria.

Type IV pili mediate twitching motility via an extension/retraction mechanism that incorporates new subunits into the base of the structure. That step requires an ATPase to extend the pilus. Then subunits are removed from the base into the cytoplasmic membrane by a distinct depolymerizing ATPase that retracts the pilus. In contrast, the archaeal motility structure rotates. Importantly for the halobacterial structure, this rotation depends on hydrolysis of ATP, further distinguishing the archaeal motility from the bacterial flagellum, which is driven by the proton motive force or, more rarely, by a sodium-motive force.

**The Name Archaellum Reflects the Sum of the Differences**

Those differences in structure, modes of action, and assembly mechanisms between the archaeal motility structure and the bacterial flagellum or type IV pilus led us to propose in 2012 a novel name for this archaeal structure—the “archaellum.” This new name emphasizes the uniqueness...
of this motility structure, setting it apart from other microbial appendages, especially the bacterial flagellum.

Archaeall can be detected directly by electron microscopy of archaeal cells or indirectly by identifying the conserved fla operon responsible for forming archaeall. Analysis of sequenced genomes shows that these structures are widespread throughout the major subdivisions of Archaea. While our proposed term, archaellum, has not been universally accepted, the name is being found with increasing frequency in research publications and reviews from archaeal-centric laboratories as well as those that focus on bacterial flagella and pili.

In crenarchaeota, the archaellum operon comprises only seven genes, but this structure still achieves the same swimming forces as the bacterial flagellum. Indeed, several hyperthermophilic archaea are among the fastest swimmers in the microbial world, according to Bastian Herzog and Reinhard Wirth at the Universität Regensburg.

It still is not known how the type IV pilus-like motor rotates. Importantly, Flal, the only ATPase in the archaellum operon, is bifunctional—essential not only for the assembly of the archaellum filament but also for the completed organelle to rotate, as revealed in a collaboration between the John Tainer laboratory at Lawrence Berkeley National Laboratory in Berkeley, Calif., and the Albers laboratory. Further, the two domains within this protein that are responsible for those two activities can be separated. An N-terminal

AUTHOR PROFILE

Albers: from Microscopy at Age 8 to an Archaea-centric World View

Sonja-Verena Albers most wanted—and her parents gave her—a simple microscope for her 8th birthday. With it, she spent hours examining water samples and plant parts she gathered during walks through nearby woods. “My father always liked to take us on these extended walks...and tried to interest us in the nature around us,” she says. “So maybe that was the initial trigger.”

Regardless of its origins, her interest in biology increased steadily as she grew older. “Biology lessons were my favorite ones during school education, so I, of course, chose the intensive biology classes for specialization at the end of high school,” she says.

“Herr Zietz” taught that intensive biology class, she recalls. “We grew our own Drosophila and looked at inheritance of certain traits. When we learned how DNA is translated into RNA and subsequently proteins, I knew I wanted to work on the molecular understanding of how a cell works. My mother tried to convince me to start working in a bank by putting advertisements for banks on my breakfast dish, but that did not change my mind.”

Albers was born and grew up in Hamburg, where her parents and younger sister still live. “Besides me, nobody in my direct family is involved in science,” she says. Her mother was a secretary, her father an engineer, and her sister a writer and Web designer.

Today, Albers, 43, is professor of biology and microbiology at the University of Freiburg where her research specializes in archaea, particularly Sulfolobus. “Archaea have intrigued me from the moment I started working with them,” she says.

“A comment that I get very often after talks I have given is ‘Oh, I had no idea that archaea can be so interesting,’” she continues. “That is a prejudice that people have generally about archaea, and I am not sure where that comes from, but of course my view of the world is very archaea-centric. I am used to working in an environment where archaea are not really taken seriously, but luckily I am mostly able to turn that into real interest.”

Albers studied biology at Julius-Maximilians-University in Würzburg and earned a Diplom (similar to a M.S.) in 1996 for thesis work in the lab of the (late) Wolfram Zillig at the Max Planck Institute for Biochemistry in Martinsried, Germany. “He was the first person in Germany who had worked on archaea,” she says. “Wolfram was very inspiring, and, although he was already 72 years old at that moment, his fire for science and his work were really astonishing.” She earned her Ph.D. at the University of Groningen in the Netherlands in 2001, and did postdoctoral research there, after which she had a Max Planck Research Group at the Max Planck Institute for Terrestrial Microbiology, Marburg, Germany. There she established her own lab studying the molecular biology of Archaea.

Albers met her husband, biochemist Chris van der Does, who is Dutch, while they were both doing their Ph.D. research. They have two sons, Niels, 8, and Jonas, 10.

Marlene Cimons
Marlene Cimons lives and writes in Bethesda, Md.
deletion mutant of FlaI, although no longer mobile, still assembles archaella filaments. This finding supports the early observation that rotation of the archaellum depends on ATP in halobacteria by the Oesterhelt laboratory.

In crenarchaeota, the motor ATPase FlaI interacts with FlaH, a nucleotide binding protein, and FlaX, forming a 30-nm ring structure that is probably the scaffold for the motor proteins, according to Ankan Banerjee, Tomasz Neiner, and Patrick Tripp, working in the Albers lab at the Max Planck Institute for Terrestrial Microbiology in Marburg.

Our subsequent structural and functional analyses identified FlaF as the first extracellular, non-archaellin, subunit of the archaellum. FlaF from *S. acidocaldarius* binds to the S-layer protein, which constitutes the sole cell envelope protein in this archaeon, leading us to propose that FlaF anchors the rotating archaellum in the archaellar cell envelope. FlaF is conserved and essential for archaellum assembly in all archaea so far, leading us to develop a schematic model for this assembly (Fig. 1).

**Many Outstanding Questions about Archaellum**

Little is yet known about how the archaellum genes are regulated except to say that their regulatory mechanisms appear to be complicated to ensure that their expression is timely.

The *S. acidocaldarius* archaellin promoter is controlled by phosphorylation. ArnA and ArnB (where Arn is an abbreviation for archaellum regulatory network) are FHA- and van Willebrand domain-containing proteins, respectively. They act as repressors of the archaellin promoter, and deleting them leads to a hyperarchaellation phenotype, according to Julia Reimann and others working in the Albers group. Both proteins are phosphorylated by specific protein kinases. Additionally, a serine/threonine phosphatase deletion mutant is highly induced. In contrast to the repressor ArnA/B, the binding of the membrane bound activator ArnR to the archaellin promoter is essential for the expression of the archaellum operon, according to Kerstin Lassak in the Albers group. In addition, the biofilm repressor AbfR1 somehow activates expression of the archaellum operon, according to collaborator Alvaro Orell.

The hydrophobic part of the type IV pilin signal peptide of various pilins in *H. volcanii* is important for archaellin expression in this organism, and this regulation is at least in part posttranslational, according to Rianne Esquivel working with Mechthild Pohlschroder at the University of Pennsylvania, Philadelphia.

In another haloarchaeon, *Haloarcula marismortui*, different archaellin genes (*flaB* and *flaA2*) are expressed when the organism grows at different salt concentrations. It appears that FlaB and FlaA2 can replace each other under different environmental stresses and thus the two archaellin proteins may be called ecoparalogs, according to Alexy Syutkin and colleagues in the Oleg Fedorov group at the Russian Academy of Sciences in Pushchino, Moscow Region. In methanogens, archaellation is influenced by various nutrients, including hydrogen, as first reported by Biswarup Mukhopadhyay working with Ralph Wolfe at the University of Illinois at Urbana-Champaign.

While the major function of the archaellum is motility, numerous reports indicate that this is not its sole function. Archaella help cells attach to various surfaces, sometimes in conjunction with pili, and also enable cells to interact with other species. Scanning electron microscopic images of *P. furiosus* by Reinhard Rachel und Gerhard Wanner show that cable-like bundles of archaella form to help cells attach to surfaces, and these structures also appear to be involved in forming biofilms and in cell-to-cell communication.

Unlike the many ways that bacteria propel themselves, to date the only motility structure identified in archaea is the archaellum. Although many archaea express type IV pili, which in bacteria can give rise to twitching movements, the pili of archaea do not appear to play any role in locomotion.

_Sonja Verena Albers is a professor at the University of Freiburg and Ken F. Jarrell is a professor in the Department of Biomedical and Molecular Sciences at Queen’s University, Kingston, Ontario, Canada._

**Suggested Reading**


Chaban, B., S. Voisin, J. Kelly, S. M. Logan, and K. F.


The Joint Genome Institute Offers Resources Beyond a Core Facility

JGI now partners with thousands of scientists worldwide to broaden and deepen its research capabilities

David Gilbert, Nikos Kyrpides, Susannah Tringe, Axel Visel, and Tanja Woyke

The U.S. Department of Energy (DOE) Joint Genome Institute (JGI) was established in 1997 to consolidate the department’s programs and resources in DNA sequencing, informatics, and technology development. Soon after, the University of California, which manages the Lawrence Berkeley National Laboratory, where JGI was first situated, leased lab and office space in nearby Walnut Creek to house JGI activities. The early focus for JGI was the Human Genome Project. After its scientists completed their sequencing of three human chromosomes, however, JGI broadened its mandate in 2004 to become a national user facility, which now boasts thousands of users worldwide.

JGI’s sequencing, synthesis, and analysis resources are managed under the auspices of the Community Science Program (CSP: http://bit.ly/CSPJGI), surveying the biosphere to characterize organisms and environments relevant to the DOE science mission, which includes interests in bioenergy, global carbon cycling, and biogeochemistry. CSP projects are selected through peer review on the basis of scientific merit, scale, complexity, and relevance. JGI provides services at no cost, so long as the data generated can be made available through its public portals.

In addition to large-scale, complex projects solicited through the annual CSP call by JGI, users also may submit projects to determine the sequences of genomes of moderate numbers of bacterial or archaeal isolates and single cells, and to analyze a panoply of epigenomes, metagenomes, or (meta) transcriptomes. Its semi-annual project request cycle enables users to obtain pilot-scale sequencing data without waiting for the full 12-month CSP cycle but often leads them to submit full-scale proposals.

JGI Researchers Generate Volumes of Data, Publish Plenty

JGI is enormously productive, not only in terms of generating DNA sequence data—in 2014, its sequencing output encompassed more than 100 trillion nucleotides. However, it also is generating many high-profile publications. Since 2011, for example, the JGI Prokaryotic Super Program, under which its Microbial and Metagenome Programs are subsumed, yielded 471 publications. Nearly 100 were published in journals with impact factors greater than 8.

JGI is the second-largest producer of prokaryotic genome sequencing projects and is the leading center for sequencing genomes of phylogenetically diverse prokaryotic species. JGI is also on the forefront of generating and publishing single-cell genomes, a cultivation-independent approach to explore the metabolic potential encoded in the many microbes that cannot be readily grown in culture.

JGI scientists developed a state-of-the-art

SUMMARY

➤ The Department of Energy established the Joint Genome Institute (JGI) in 1997, consolidating departmental DNA sequencing activities, technology development, and bioinformatics resources that took shape during its participation in the Human Genome Project.

➤ Now managed under its Community Science Program (CSP), JGI projects focus mainly on microbial communities, fungi and plants related to bioenergy, global carbon cycling, and biogeochemistry.

➤ JGI is the second-largest manager and backer of prokaryotic genome sequencing projects, and is the leading center for sequencing genomes of phylogenetically diverse prokaryotic species.

➤ JGI continues to broaden the resources it provides through CSP, to expand its scientific and technologic capabilities, and to support projects that cross phylogenic boundaries.
pipeline for isolating individual cells and amplifying their genomes to produce the amount of DNA necessary for genome sequencing. With this pipeline in place, JGI users have produced high-impact studies of novel microbes, and success with this approach built demand for single-cell genomes requested through the annual CSP program. Starting in 2015, JGI began offering single-cell sorting, genome amplification, and sequencing to JGI users as part of the Small-Scale Microbial/Metagenome call for proposals, which supplements the annual CSP program.

**JGI Is Broadening User Services through CSP**

For those who submit proposals through CSP, JGI offers two types of microbial assembly products. One, based on the Illumina sequencing platform and called "Minimal Drafts" (http://jgi.doe.gov/collaborate-with-jgi/product-offerings/), is intended for both isolates and single cells, and the second, which is Pacific Biosciences (PacBio)-based, is called “Improved Drafts” and is restricted to isolates.

Beyond this genome assembly menu, the microbial product catalog also offers resequencing of bacterial and archaeal genomes. The output includes a detailed summary report of identified SNPs, indels, and structural variants. RNA-seq for prokaryotes is another key product, used for both annotation improvement and transcript counting. For functional characterization of prokaryotes at the level of the epigenome, DNA modification plays an important role in DNA restriction systems involved in bacterial immunity, while several types of modifications are involved in gene regulation.

Two recent product additions are Methyl-Seq and Tn-Seq, both of which fit with the JGI 10-Year Strategic Vision (http://bit.ly/JGI-Vision) to supplement sequence data with functional information. While Tn-Seq is performed on the Illumina platform, Methyl-Seq for microbial isolates exploits the PacBio single-molecule sequencing platform, which allows robust detection of 4-methylcytosine, 5-methylcytosine, and 6-methyladenine. Thus far, JGI has interrogated more than 300 bacterial genomes for these modified bases, identifying novel DNA modifications and methylase specificity, adding new insights into restriction and gene regulatory systems.

The JGI Metagenome Program produces high-throughput sequencing of microbial community nucleic acids for phylogenetic and functional characterization. Current metagenome standard draft protocols call for a single 300-bp insert Illumina library, which is sequenced on the HiSeq platform with paired 150-bp reads. Assembly of the resulting data typically results in draft-level coverage of one or more dominant genomes, but for complex environmental samples such as soil or sediment the degree of assembly is still low. For a few samples, these data are supplemented with Illumina long mate-pair reads or PacBio data, producing larger contigs and scaffolds.

Metagenome minimal draft projects are treated similarly, with construction of 300-bp insert Illumina libraries generated for each sample, preferably in plate format, and sequenced in pools of 6–12 libraries per HiSeq channel, also with paired 150-bp reads. Assembly is performed for all these datasets, typically producing minimal assembly for complex environmental samples but a high degree of assembly for low-diversity samples such as those from hot springs or laboratory enrichments.

Metatranscriptome (RNA) sequencing is a steadily growing proportion of the JGI service portfolio, due to reductions in the amount of input RNA required and improvements in protocols for removing ribosomal RNA. RNA samples from collaborators are depleted of ribosomal RNA, and then used for constructing strand-specific Illumina libraries, which are sequenced with paired 150-bp reads. This process leads to de novo assembly as well as mapping to one or more references, including matched metagenomes or relevant isolate genomes.

JGI continues to make progress in sequencing, assembling, and annotating metagenome data from analyses of highly complex microbial communities found in soils and sediments. Many projects target terrestrial environments across the globe, ranging from permafrost to tropical soils and from wetlands to desert crusts, including a number of experimentally manipulated sites investigating the impacts of climate change. Many of these metagenome projects are augmented by isolate genome sequencing, single-cell genome sequencing, and 16S-ribosomal RNA tag sequencing. Custom analyses, including via co-assembly of sequences from several metagenome, metatranscriptome, or single-cell samples, are
available for such projects. Shifting focus from terrestrial carbon cycling, the most recent JGI call for proposals sought sequencing projects studying extreme environments, including the deep subsurface.

**Plans Call for Expanding Metabolomics Capacity**

Understanding microbial metabolism, which is central to understanding biogeochemical cycles, plant-microbe interactions, and enabling sustainable bioenergy, is an emerging area of emphasis for JGI. Metabolites of interest range from simple catabolic substrates to complex secondary metabolites with largely unknown functional roles in microbial communities.

Integrating metabolomic and genomic technologies provides a gateway for discovering and annotating gene functions. For example, correlating metabolites with transcriptomics can suggest gene functions, as can correlating genes and metabolites across strain collections. Definitive functions can be determined by integrating metabolomics with DNA synthesis or mutagenesis, enabling JGI users to discover the genetic determinants of key metabolic functions.

Consistent with JGI’s 10-year strategic vision to further advance the frontiers of genomics paired with molecular characterization, JGI issues an annual collaborative call with Pacific Northwest National Laboratory’s Environmental Molecular Sciences Laboratory (EMSL): http://bit.ly/JGI-JECSI.
In house, JGI has also established a mass spectrometry-based metabolomics facility to develop untargeted metabolite profiling approaches. This approach is enabling users to identify unexpected soluble metabolites in cell extracts of bacteria. Such findings can shed light on the functions of unknown genes.

One new approach for discovering gene functions uses Tn-mutant libraries and comparative genomics to annotate both enzymes and transporters. Because a single unknown metabolite may be associated with multiple genes, this approach could have a significant impact on genome annotation. Secondary metabolites are of particular interest because they account for a large fraction of many microbial genomes, and yet both their structures and functions are poorly understood. As an initial focus, JGI is targeting crude plant biomass/lignin hydrolysates for bioenergy applications and soil/plant/microbial extracts for carbon cycling experiments.

**Amid Expansion, Resources To Assure Data Quality**

All JGI microbial and metagenome sequencing projects are subject to quality controls using state-of-the-art tools. For example, assembled datasets are fully annotated using the Integrated Microbial Genomes (IMG) annotation pipeline, which is a genomics platform for comparing sequences of public available bacterial and archaeal genomes and asking questions at the scale of genes, pathways, and organisms (http://www.ncbi.nlm.nih.gov/pubmed/24165883). There is a comparable system for metagenome analysis (IMG/M: http://www.ncbi.nlm.nih.gov/pubmed/24136997).

To make these and other analytical tools more accessible to those who need them, JGI convenes periodic workshops on microbial genomics and metagenomics (http://mgm.jgi.doe.gov/). Each workshop includes seminars and extensive tutorials on how to use IMG systems for comparative analysis of genomes and metagenomes.

Many researchers now can sequence genomes from isolates or single cells, as well as metagenomes, in their own core facilities or labs, but may lack the capacity to analyze datasets. The IMG/M-Expert Review (ER) platforms fill this void and allow users to upload their data, carry out comparative genomic analyses, provide annotations, and export results in a secure environment.

The JGI Prokaryotic Super Program also manages and maintains the Genomes OnLine Database (GOLD: http://bit.ly/JGI-GOLD), a resource for comprehensive access to a metadata-rich catalog of genome and metagenome sequencing projects from around the world.

**Sequence-Enabled Science, Crossing Phylogenetic Boundaries**

JGI scientists are sequencing nucleic acids from individual microbes, targeted populations, and entire communities to understand the evolution and global roles of microbial life on Earth. Key scientific foci for JGI include expanding the catalog of phylogenetic and functional diversity, characterizing and predicting the roles of microbes in global nutrient cycles, and understanding symbiotic relationships among plants and their microbial partners. Projects addressing these aims are not only using many different sequencing approaches, but are combining them in novel ways—for example, mapping metatranscriptome data to isolate genomes or using single-cell genomes to bin metagenome data phylogenetically.

The JGI has been a pioneer in sequencing of phylogenetically diverse microbial genomes, starting with the *Genomic Encyclopedia of Bacteria and Archaea* (http://bit.ly/JGI-GEBA) initiative and continuing with GEBA-Cyano for the cyanobacterial tree of life, GEBA-RNB for root-nodulating bacteria (http://bit.ly/GEBA-RNB), and the Genomic Encyclopedia of Type Strains projects (http://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.1001920). The development of a robust single-cell genomics pipeline led to the GEBA-Microbial Dark Matter Project, exploring 201 genomes from uncultivated candidate phyla of bacteria and archaea. Other examples leveraged metagenome assembly tools to reconstruct more than 90 partial genomes from cow rumen and to explore the prevalence of alternative genetic codes in the wild.

Integrative studies are lowering the barriers between domains and programs. The project studying plant-microbe interactions in the rhizosphere, for example involves diverse JGI capabilities and data types, including microbial community profiling, microbial isolate and single-cell
sequencing, and metagenomic and metatranscriptomic sequencing.

Another example is a project to identify and characterize the phylogenetically diverse glycosyl-hydrolase family 1 (GH1) of enzymes. This effort includes mining of JGI metagenomic and single-cell sequence data for GH1 genes, phylogenetic analysis of GH1s, DNA design and synthesis of hundreds of GH1 constructs, expression of synthetic GH1 constructs in laboratory hosts, and metabolomic functional assays to link specific enzymes to functional characteristics. This type of complex large-scale project, requiring a diversity of “omics” capabilities and analyses, exemplifies an increasing fraction of the work that JGI undertakes.

**What Lies Ahead**

These examples describe some of the expanding experimental and computational capabilities and organizational changes that are enabling users to carry out more large-scale environmental and systems biological studies. Changes in technologies offered by JGI reflect its evolution from being primarily a production sequence facility to becoming a next-generation genome science user facility, offering diverse capabilities that complement large-scale, state-of-the-art DNA sequencing. These combined capabilities are enabling JGI to meet the expanding needs of its users and reinforce its position as the leading user facility in energy and environmental genomics.

Through its Emerging Technologies Opportunity Program (ETOP), JGI is developing new capabilities, including functional trait-based sorting of microbial cells, single-cell approaches to metagenomics, high-throughput recovery of microbial genomes from metagenomic datasets, and a microfluidic platform for high-sensitivity DNA library construction from microbial cells and genomic DNA. ETOP plans to offer these new resources to the growing JGI user base.

Each year, JGI convenes more than 400 current and prospective users for its Genomics of Energy and Environment Meeting, the 11th of which will be held 21–24 March 2016. Additional details about these meetings are available at http://usermeeting.jgi.doe.gov/.

David Gilbert, Public Affairs Manager; Nikos Kyrpides, Prokaryote Super Program Head; Susannah Tringe, Metagenome Program Head; Axel Visel, User Programs & Strategic Planning Head; and Tanja Woyke, Microbial Program Head, are at the Joint Genome Institute, Walnut Creek, Calif. The work conducted by the U.S. Department of Energy Joint Genome Institute, a DOE Office of Science User Facility, is supported under Contract No. DE-AC02-05CH11231.

**Suggested Reading**


Enquist Assumes ASM Presidency

Starting 1 July 2015, ASM welcomes Lynn Enquist, Henry L. Hillman professor of molecular biology at Princeton University and the Princeton Neuroscience Institute, as its new president. He was elected in early 2014 and has served as president-elect for the past year. An elected fellow of the American Academy of Microbiology and the American Association for the Advancement of Science, Enquist is an expert on neurovirology and molecular genetics.

His research interests include genetics and molecular biology of DNA viruses with a special emphasis on neurotropic alpha herpes viruses. His current work is devoted to understanding how viruses invade and cause disease in the nervous system.

Enquist has already been involved in important roles for the Society. Recently, he has been active in the ASM Futures Project, which is exploring possible changes to organizational and governance structures. He also is a member of the search committee for ASM’s new ED/CEO. “I am very excited to be President during this time of change for ASM and am looking forward to working with members as we move forward to the future of the Society,” says Enquist. He has also served on the editorial board and held the position of editor-in-chief of the Journal of Virology.

Enquist has also served as an executive scientist at Molecular Genetics, Inc., one of the first biotech companies, led efforts in designing novel applications of viruses for DuPont corporate research, and was a senior research fellow for DuPont Merck Pharmaceutical Company. Enquist has also been a member of the National Science Advisory Board for Biosecurity and of the American Association for the Advancement of Science board of directors, and has served as President of the American Society for Virology. He was appointed as a commissioner for the New Jersey Cancer Commission, where he played a leadership role in state funding of cancer research and education. Additionally, he has been elected to the American Academy of Arts and Sciences.

ASM Partnerships Update

ASM is pleased to announce the following new partnerships and alliances.

**ASM Partners with Bill & Melinda Gates Foundation on ICAAC.** ASM has received a $161,460 multiyear grant from the Bill & Melinda Gates Foundation to help support the research being presented at ASM’s Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC). Through their Global Health Division, the foundation will not only partner with ASM to host joint sessions during the conference, but also provide travel awards for scientists from low income countries through The Gates Travel Award program.

**ASM and FEMS Expand Collaborative Relationship.** Representatives of ASM and the Federation of European Microbiological Societies (FEMS) recently met to reaffirm the common interests shared by the two organizations and their commitment to building collaborative relationships to strengthen the practice, education, and advocacy of the science. As a result of this meeting, the organizations agreed to broaden and intensify joint activities over the next three years. In June ASM attended the 2015 FEMS Congress in Maastricht, The Netherlands to formally sign a Memorandum of Understanding between the organizations. Collaborative activities outlined in this agreement include:

- Support for the Mäkelä-Cassell Travel Award for Early Career Scientists
- Hosting joint sessions at respective annual meetings
• Exploration of co-publishing a Colloquium Report
• Exploration of an international microbiology recognition program
• Consideration of joint programs related to microbiology education

ASM teams up with Nesta to promote Longitude Prize in Antibiotic Resistance. ASM has agreed to partner with Nesta to promote the Longitude Prize to ASM Membership. The Longitude Prize is looking to help tackle the problem of antibiotic resistance with a £10 million prize fund for a diagnostic tool that can rule out antibiotic use or help identify an effective antibiotic to treat a patient. Register now and help to change the world (www.longitudeprize.org).

ASM, CAP, and CLSI Collaborate to Prepare Materials to Address New Regulations in the Laboratory. Representatives from ASM, the College of American Pathologists (CAP), and the Clinical and Laboratory Standards Institute (CLSI) have jointly prepared materials for laboratories to use as a guide in development of an antimicrobial susceptibility testing (AST) Individualized Quality Control Plan (IQCP) for a commercial automated AST system.

The IQCP is the Clinical Laboratory Improvement Amendments (CLIA) Quality Control (QC) policy that will become effective as an alternative QC option for all laboratory tests on 1 January 2016. The materials developed AST IQCP’s that align with the specific needs of individual labs. Specifically, the following materials are available:

• Template (PowerPoint®) that describes the components that should be included in an IQCP for a commercial MIC AST system
• Example of a completed IQCP (tabular format)
• Listing of Q&A’s

Visit http://clinmicro.asm.org/iqcp to access these resources.

ASM Meetings and Conferences

ASM Microbe 2016: A Unique Forum Unlike Any Other. Integrating ASM’s General Meeting and ICAAC, the all-new ASM Microbe 2016 (16–20 June 2016, Boston, Mass.) is the only meeting where you can explore the full scope of microbiology—from basic science to translational and clinical application. Covering seven programs tracks including a new Profession of Microbiology Track, this meeting is the place to see the best microbial sciences in the world, interact with multidisciplinary microbiologists, and meet leading product and service providers. It also offers targeted transdisciplinary sessions, topic-based networking opportunities, track-specific lounges, and more. For more information on this one-of-a-kind event, visit www.asm.org/microbe2016.

ICAAC/ICC 2015: Time is Running Out to Save on Registration. Discounted registration rates for the joint ICAAC/ICC 2015 meeting (17–21 September 2015, San Diego, Calif.) expires on 6 August 2015. Register now before the rates go up! Offering unmatched opportunity for discussion, cross collaboration, and advancement, this joint meeting will debut advances in drug development and infectious disease research and examine antibiotic resistance, new antibiotic development and antibiotic stewardship. This will be 2015’s principal meeting to better improve the diagnosis, prevention, and treatment of infectious diseases around the globe. For more information and to register, visit www.icaac.org/icaac-icc.

2016 ASM Biodefense and Emerging Diseases Research Meeting: Abstract Submission Opens September. Recognizing that emerging infectious diseases serve as a paradigm for handling the public threat of bioterrorism, the ASM Biodefense and Emerging Diseases Research Meeting (8–10 February 2016, Arlington, Va.) brings together researchers and policymakers to
review the current state of bioterrorism agents, detection and diagnostic procedures, animal and plant pathogens, and global surveillance. Attend this meeting for powerful plenary sessions and symposia exploring bioterrorism research and policy. Abstract submission opens 8 September 2015. To learn more, visit www.asm.org/biodefense2016.

**Upcoming ASM Conferences.** ASM conferences address the needs of the diverse scientific interests of microbiologists by providing a forum for international groups of scientists, ranging from 100 to 400 participants, to discuss their specific area of concentration. Mark your calendar for these upcoming ASM conferences. For more information, visit www.asm.org/conferences.

- **ASM Conference on Pseudomonas 2015** (8–12 September, Washington, D.C.)
- **7th ASM Conference on Biofilms** (24–29 October 2015, Chicago, Ill.)
- **4th ASM-ESCMID Conference on Methicillin-resistant Staphylococci in Animals: Veterinary and Public Health Implications** (2–5 November 2015, Chicago, Ill.)
- **13th ASM Conference on Candida and Candidiasis** (13–17 April 2016, Seattle, Wash.)
- **AMS Conference on Streptococcal Genetics** (31 July–3 August 2016, Washington, D.C.)
- **6th ASM Conference on Beneficial Microbes** (9–12 September 2016, Seattle, Wash.)
- **5th ASM Conference on Salmonella** (Fall 2016)
- **ASM Conference on Antibacterial Development** (11–14 December 2016, Washington, D.C.)
ASM Public Affairs

ASM Comments on the 21st Century Cures Act

ASM sent comments on the 21st Century Cures Initiative to the House Committee on Energy and Commerce. The bill aims to stimulate both innovation in biomedical research and the development of new medical treatments and cures. The ASM applauded the Act’s $10 billion increase in National Institutes of Health (NIH) funding over five years, which is intended to be mandatory funding outside the annual appropriations process. This proposed increase would provide needed additional support for the NIH, which funds much of the Nation’s biomedical discoveries. The bill’s authorization of an additional $1.5 billion in discretionary funding for each of the next 3 years will help to set NIH on a path to growth after years of stagnant funding. The ASM made recommendations on the $10 billion NIH Innovation Fund, the Biomedical Research Working Group, travel for NIH supported scientists to scientific meetings and conferences, and interagency cooperation between the NIH, Food and Drug Administration and the Centers for Disease Control and Prevention on the development of new antimicrobials. To read the ASM letter, go to https://www.asm.org/index.php/public-policy-2/statements-testimony/137-policy/documents/statements-and-testimony/93456-lp-4-15) and is currently working with ASM to identify expert volunteers to assist with development of a protocol for validation of an endoscope culturing method. To see the FDA’s most current documents on infections associated with reprocessed duodenoscopes, please go to http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/ReprocessingofReusableMedicalDevices/ucm436580.htm.

ASM Joins S-FAR Webinar on S.185, the PATH Act

On 7 May, ASM joined the U.S. Stakeholder Forum on Antimicrobial Resistance (S-FAR) in a webinar with staff from the offices of Senators Orrin Hatch and Michael Bennet, who introduced the Promise for Antibiotics and Therapeutics for Health (PATH) Act, S. 185. This legislation amends the Federal Food, Drug, and Cosmetic Act to require the Food and Drug Administration to establish a program to approve an antibacterial drug intended to treat a serious medical condition and to address an unmet medical need within an identifiable limited population as a limited population antibacterial drug. S-FAR was convened on the principle that U.S. government strategies to address antimicrobial resistance should involve sustained engagement with experts and stakeholders throughout the policy development and implementation process. To read more about S-FAR, go to http://www.s-far.org/.

FDA and CMS Form Task Force on LDT Requirements

ASM staff have been monitoring the FDA “Framework for Regulatory Oversight of Laboratory Developed Tests (LDTs)” since it was issued in October 2014 (http://www.fda.gov/downloads/medicaldevices/device regulationandguidance/guidancedocuments/ucm416685.pdf). Under the proposed framework, FDA would oversee the quality of these tests, with the Centers for Medicare and Medicaid Services (CMS), which regulate the laboratories through the Clinical Laboratory Improvement Amendments (CLIA), to coordinate efforts, FDA and CMS have established an inter-
agency task force that will continue and expand collaboration related to oversight of LDTs and will be comprised of subject matter experts from each agency. Read more about it at http://blogs.fda.gov/fdavoice/index.php/2015/04/fda-and-cms-form-task-force-on-ldt-quality-requirements/.

Education Board

Fellowship Opportunity for Undergraduate STEM Faculty

Early-career (and future) undergraduate STEM educators are encouraged to apply for a 2015 ASM-LINK Undergraduate Faculty Research Initiative (UFRI) Fellowship. This new professional development resource trains STEM faculty to develop undergraduate research programs by initiating successful research partnerships. As part of the fellowship, ASM LINK will provide travel subsidies of up to $2,000 to (i) increase participation of undergraduate STEM educators at seven eligible ASM-sponsored research conferences, (ii) encourage networking and collaborations with potential research partners, and (iii) access resources and mentoring to advance undergraduate research programs.

Fellowship applications are accepted on a rolling basis for each of the seven eligible ASM conferences. Deadlines are 24 and 31 August 2015, respectively, to be considered for UFRI’s Fellowships for the 2015 ASM Conference on Biofilms and the 2015 ASM-ESCMID Conference. To learn more, visit http://www.asmlink.org/ufri.

ASM Science Teaching Fellowship Program

Graduate students, postdoctoral fellows, and early-career scientists are encouraged to apply for the 2014–2015 ASM Science Teaching Fellowship Program, a four-month online training experience that guides doctoral-trained participants in understanding the essentials of science teaching positions at non-doctoral institutions (community colleges, minority-serving institutions, regional or state colleges, and other primarily undergraduate institutions). Program activities combine structured mentoring with in-depth webinars, pre- and post-webinar assignments, and a highly interactive community of practice, all focused on four areas: teaching science to undergraduates, curriculum and course design and assessment, student-centered learning, and students as research collaborators. The experience is fast paced, intense, and interactive, featuring practical examples in microbiology education. To join the program, apply by 12 September. Learn more at http://www.facultyprograms.org/index.php/stf-program.

Obituaries

Anne M. Dranginis

The yeast community mourns the passing in April of Anne Dranginis, who remained cheerful and collegial through 4 years of cancer treatment. Anne’s strength was in her ability to identify and explain basic phenomena that were overlooked or considered to be “too difficult” to tackle. As a result, her output was modest, but her citation rate was remarkable. Two of her findings merit special attention. In the 1980s and 1990s, she was interested in how mating type in yeast regulates expression of specific genes: some are expressed only in one mating type or the other, some genes are expressed in all haploid cells, and some only in diploids heterozygous at the MAT locus. As a result, she showed that cell type could be determined according to which DNA binding proteins dimerize. This mechanism has become a key to the combinatoric models of regulation of cell fate in evo-devo models. Anne also helped blaze a trail that multiple other investigators followed with her discovery of Flo11 as the cell surface flocculin that orchestrates the social lives of yeast cells. Flo11 is required for formation of biofilms, mats and pellicles. Her studies elucidated the molecular architecture of this key cell-cell adhesion molecule, including a new type of lectin domain and that FLO11 has the largest known promoter in yeast (and thus perhaps the most complex regulation).

Anne was a remarkable mentor at all levels. One of her mentees writes: “I met Anne 16 years ago as an undergraduate researcher. As a shy student, establishing a rapport with faculty was never easy; however, I was immediately drawn to Anne’s easygoing and pleasant disposition. I never imagined the important role and impact Anne would play in my career development at that time. Anne was one of my dissertation and junior faculty mentors. I could always rely on her for career advice, letters of support, and experimental ideas. Her scholarship, integrity, and teaching inspired many students and myself.” She brought her vision to a broader stage via the Asilomar Fungal Genetics meetings and community. It is no surprise that she was awarded the St. Johns University Excellence in Teaching Award at the graduate level.

Anne received her B.S. degree in zoology from the University of Massachusetts, and her Ph.D. in cellular and molecular biology from the University of Michigan, where she focused on am-
ylase gene structure and expression. After her work at NIH, Anne moved to join the faculty at St. John’s University in 1992, becoming full professor and assistant chair of her department until her retirement shortly before her death. Dranginis was elected a Fellow of the American Association for the Advancement of Science in 2013. She was also a public speaker for science, and was published in the New York Times op-ed section on critical issues, including the delay in studying hormone replacement therapy and the scarcity of key chemotherapeutic drugs due to commercial considerations. With grace and wit she exemplified all that is good in science, inspiring others as a consummate, gifted educator. Her guiding voice and insightful science will be broadly missed.

Giuseppe Bertani

Professor Giuseppe Bertani (Joe to friends) died on April 7, 2015 at the age of 91 in Pasadena, CA. As a pioneering microbial geneticist, his insights helped to develop both modern microbiology and the molecular biology of today. Born in Como, Italy, Joe was raised in Milan, where he earned his doctorate in zoology. After postgraduate studies in Naples and Zürich, he arrived at the Cold Spring Harbor Laboratory (CSHL) in October 1948 as a Carnegie Fellow working in Milislav Demerec’s group. Here, he shifted his focus to bacterial genetics and was soon measuring reverse mutation rates in a streptomycin-dependent mutant strain of Escherichia coli after exposure to radiation and chemical agents; in fact, these experiments preceded what would later become the Ames test. Most importantly, it was here that Joe was shown phage plaques for the first time by his friend Gus Doermann, who was working on phage T4, and that he first encountered lysogeny.

Joe attended Max Delbrück’s phage course at CSHL in 1949, after which he joined Salvador Luria at Indiana University in Bloomington. Here he began studying lysogeny, although at first Luria was somewhat reluctant. Using what he called a “modified single burst technique” Joe demonstrated that phage production by a lysogen was discontinuous, involving rare, large bursts of phage. He went on to characterize the establishment of lysogeny, the state of the prophage, and superinfection immunity. As it turned out, the L-bonne strain Joe was using produced P1, P2, and P3. It was P2, the noninducible phage, which was to become his primary phage of study. During these studies Joe composed the now ubiquitous LB medium, which subsequently has been referred to as Luria broth, Lennox broth, or Luria-Bertani medium. For the historical record, Joe pointed out that the abbreviation LB was intended to stand for “lysogeny broth.” In addition to his lysogeny work, Joe’s discovery in 1953 of “host-controlled variation,” together with Jean Weigle, ushered in our understanding of host restriction and modification, which influenced the discovery of restriction enzymes 15 years later.

Joe remained with Luria after the lab moved to the University of Illinois in 1950, where he met and married Betty, and then in 1954 Joe joined the laboratory of Max Delbrück at Caltech. In 1957 Joe took up a professorship in the medical school at University of Southern California in Los Angeles, where Werner Arber joined him as a research associate from 1958 – 59.

In the early 1960s Joe was appointed professor in microbial genetics at the Karolinska Institute and studies of phage P2 became the focus of the Bertani lab. In these years a steady stream of postdoctoral fellows filled his laboratory in addition to his students and many distinguished visitors. In addition to his obligations at the Karolinska Institute he was also responsible for the advanced teaching of microbiology at the University of Stockholm. His influence on the scientific community in Sweden was significant and his work was recognized by Uppsala University where he received an honorary doctorate in 1982. During this time he also participated in establishing the European Molecular Biology Organisation (EMBO). In 1981 he returned to California to take up a position at the Jet Propulsion Laboratory (JPL) in Pasadena, where he studied the genetics of methanogenic bacteria and described a curious phenomenon of transduction. After formally retiring from JPL in 1991, Joe continued as a voluntary scientist in the Division of Biology at Caltech.

Joe was highly critical but generous when it came to publishing. He rarely put his name on his students work when they were ready to publish their results. Joe Bertani was an outstanding scientist with a philosophical touch, belonging to that dwindling group of pioneers in microbial genetics with roots in the legendary Phage Group. We thank him for taking us on a marvelous journey in science, with him as our guide, and for his friendship. Our thoughts are with his wife Betty, their sons Christofer and Niklas, and their families.

Richard Calendar
University of California, Berkeley

Elisabeth Haggård-Ljungqvist
Stockholm University

Bjorn H. Lindqvist
University of Oslo

Steven E. Finkel
University of Southern California
**Microbe Mentor**

I am about to graduate with a Ph.D., and would like to eventually find a job in industry. How to I structure a professional resume for applying to industrial, non-academic positions?

Excellent question! The fact that you understand that there even is a difference between a curriculum vitae, or CV, and an industrial/professional resume has you ahead of the game. Quick review: an academic CV catalogs a person’s academic career, thus contains the full reference for every publication and presentation given, all awards, honors, committee membership lists, etc. A CV can encompass decades’ worth of a career. The content and format are primarily tailored to highlight a person’s overall experience, and are reviewed by peers who generally understand the technical verbiage used in publication and presentation titles.

In contrast, a professional resume summarizes the most recent years of a professional life (often not going back more than 10 years, unless something is particularly relevant). The format and content of a resume are tailored to specifically highlight how closely an applicant matches a specific job posting. Resumes are often reviewed by a Human Resources Department who will likely not be fluent in technical verbiage.

So, how to create a resume when you are at the start of your career? Fortunately, most senior-level graduate students actually have experience necessary for nonacademic employment and do not even realize it! Start looking at your everyday activities from the perspective of somebody in a professional environment. Read lots of job postings in your field—you will start to notice common elements, such as “Must be able to multitask multiple projects, demonstrate the timely delivery of high-quality work products, and maintain corporate health and safety protocols.” Now, think of how your graduate-school responsibilities can demonstrate how you’ve done this.

The next step is formatting your resume. A quick search of the internet shows many formats, each with pros and cons. In general, however, keep the following in mind:

- **DO** use a clean and uncluttered format.
- **DO** use bullets, which allow for the reader to quickly scan the document, get interested, and then slow down to read it in more detail.
- **DO** treat the page as real estate; blocks of blank space are wasted opportunities to mention something that will make you stand out.
- **DO** use one font type and size; this will allow for easier reading, and will reduce the chance

<table>
<thead>
<tr>
<th>Everyday activity description</th>
<th>Description rephrased for professional resume</th>
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<tr>
<td>Work with younger graduate students or undergraduate researchers in your lab</td>
<td>Mentor junior personnel</td>
</tr>
<tr>
<td>Develop, modify, or follow laboratory or experimental protocols</td>
<td>Design, evaluate, and follow technical protocols</td>
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<tr>
<td>Maintain coursework and teaching responsibilities while also making progress on your dissertation</td>
<td>Balance and prioritize multiple deliverables</td>
</tr>
<tr>
<td>Operate scientific laboratory instruments (GC, GC-MS)</td>
<td>Operate and maintain sensitive and technical equipment</td>
</tr>
<tr>
<td>Write for scientific publications, draft grants, present at conferences</td>
<td>Possess excellent technical communication skills</td>
</tr>
<tr>
<td>Teach undergraduate labs/courses or write articles for a general audience</td>
<td>Possess excellent non-specialist communication skills</td>
</tr>
<tr>
<td>Follow protocols to safely handle chemicals, lab equipment, or cultures, and enforce the use of lab coats and safety glasses during experiments</td>
<td>Adhere to health and safety regulations such as enforced use of personal protective equipment (PPE)</td>
</tr>
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(As a rule, you should use the past tense for former positions and the present tense for your current work.)
of problems when electronically uploading a resume to a company’s employment website.

- **DO** write a concise paragraph for the top of the page. This should summarize how you meet the job requirements, and stress your unique skills and achievements.
- **DO** follow this with sections for education, job experience, professional memberships, certifications, etc. Use the order of these items to stress relevance to a particular job posting.
- **DO** include key words from the job posting. Resumes are often first reviewed by a computer, ranking them based on the number of matches to a list of key words. If the resume passes this test it will be forwarded to an actual human.
- **DO** keep in mind your resume will likely be judged by a person not trained in technical verbiage.
- **DO** customize your resume for each job you are applying for.
- **DO** include a link to your LinkedIn profile.
- **DO** build a very robust LinkedIn profile—list every publication and presentation. Prospective employers can only judge you on what you give them—and many will check your profile before deciding to contact you.
- **DO** stress any professional certifications, licenses, etc.
- **DO** have a mentor read your resume before submitting.
- **DON'T** waste space on your name, address, etc.; put this in a header
- **DON'T** include your hobbies or outside interests, unless they have contributed directly to your professional development. Employers don’t care that you enjoy photographing puppies on your weekends.
- **DON'T** include information about race, age, marital status, or nationality (unless the job specifically states that only U.S. citizens can be considered, due to mandatory security clearances, for example).
- **DON'T** overuse italics or boldfacing—non-uniform formatting makes a document harder to read.
- **DON'T** use the word “research,” unless you are specifically applying for an R&D position. Refer to dissertation “projects” or “deliverables.”

Always keep in mind that employers care about what you can do for them—not what you want—so don’t include a statement about your goals (“... wanting to become a fermentation specialist ...”). They care what you bring to the table for them to use. Once you’ve proven yourself at your job, then you can start telling your employer how you would like to develop as your career progresses.

**Eleanor M. Jennings, M.S., Ph.D.**

*Eleanor Jennings is a Principal Microbiologist at Total Environmental Concepts, Inc., an environmental consulting firm located in the Washington, D.C., area. She has worked on contaminant remediation projects on multiple continents, and currently serves as the U.S. science advisor to the National Science and Engineering Council of Canada. She is also the Chair of the ASM Career Development Committee and is on the ASM Membership Board.*

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REVIEWS

BOOK

Principles of Microbial Diversity

The term “Microbial Diversity” initially sounds like a subdiscipline of microbiology, and therefore an even narrower subdiscipline of biology. However, this innocent-sounding term includes the vast majority of species diversity on the planet, and together these species represent a far broader range of physiological diversity than that found in the small fraction of species that are macroscopic. So, writing a microbial diversity textbook is no small feat. Principles of Microbial Diversity, by James W. Brown, is designed as a text for an advanced undergraduate course in microbial diversity. The emphasis on diversity is unique—most microbiology textbooks focus on the physiology of microbes and their role in human health and the environment, and pay less attention to general issues of microbial diversity and the understudied groups of environmental microbes known mainly from gene surveys. Principles of Microbial Diversity attempts to give students an appreciation for the full diversity of microbes, and provides descriptions for many of the often-neglected bacterial phyla. One of the challenges of this approach is presenting the essentials of such a vast and ever-changing field in a finite form, accessible to advanced undergraduate students. Brown has succeeded in writing a clear and concise introduction to this subject.

This is a nonintimidating textbook with 416 pages, available in paperback. Overall, the structure is logical (though I noticed a few minor idiosyncrasies in which the material seemed out of order to me). The book is organized into four main sections: (I) Introduction to Microbial Diversity, (II) The Microbial Zoo, (III) Microbial Populations and (IV) Conclusion: The Phylogenetic Perspective.

The introductory section provides a basic discussion of diversity concepts, a historical perspective of biological diversity, and a practical phylogenetic framework for studying microbial diversity, including an introduction to the three-domain tree of life. The historical perspective is useful, and noting the false divide between prokaryotes and eukaryotes will please other microbial chauvinists like me. The modern perspective of diversity is presented in the context of previous concepts like the Whittaker five-kingdom system. While most college students are presumably no longer taught the old system, this does provide an important historical perspective on how drastically our understanding of diversity has changed in a relatively small time. The section on phylogenetic methods might not satisfy a specialist, but seems appropriate for an upper division undergraduate class: it only covers neighboring joining trees in detail, but at least briefly describes more advanced evolutionary models and methods such as maximum likelihood, parsimony, and Bayesian analysis. The treatment of horizontal gene transfer is thoughtful, though brief. I would expect this introductory section to have a more thorough treatment of difficulties with the species concept in bacteria (though maybe it is wiser to simply admit that it is somewhat arbitrary and move on). This topic gets visited much later, in Chapter 14 (in the section subtitled, “How much of the microbial world do we know about?”).

The second unit, comprising the bulk of this textbook, is devoted to a tiptoe through the taxa of the “Microbial Zoo.” Thirteen bacterial phyla are covered over six chapters and “Bacterial Phyla with Few or No Cultivated Species,” such as Acidobacteria, Verrucomicrobia, and various others are covered in an additional chapter. The groupings within some of these chapters are arbitrary, for example Spirochetes and Bacteroidetes are lumped together for superficial reasons, and Deinococcus-Thermus is included with Chlamydiae and Planctomycetes, rather than with the other thermophilic lineages. This is a natural problem given the lack of clear superphylla in the bacteria tree and the desire to impose some sort of organizing principle. Anyway, the content of these chapters is good: avoiding an excruciating level of detail, the author touches on the most salient aspects of each phylum.

This textbook is ambitious in its attempts to describe most of the major microbial phyla. Given the magnitude of the task and how quickly the field changes, this book is bound to be incomplete and certain parts (such as the description of various uncultured taxa) are destined to be out of date soon after publication of any edition. Some of the statements made in this section are already in need of revision. Many more isolates from groups that previously had few or no cultured representatives have been obtained: Acidobacteria isolates are becoming more common; OP10 now has a cultured representative; Proteobacteria now has a zeta
class; the NC10 candidate division, which includes the remarkable *Methylobacterium oxyfera*, is also worthy of mention. Archaea are summarized in just one chapter. This chapter mentions Nanoarchaeota and Koryarchaeota but not Thaumarchaeota, a candidate phyla that includes the archaean ammonia oxidizers, a group with a very important environmental function. Eukaryotic microbes are also covered in a single chapter. Perhaps the saddest omission is that fungal diversity is limited to one short paragraph about *Saccharomyces cerevisiae*. Similarly, the chapter on viruses (which includes a section on prions) is very short and doesn’t even hint at their vast diversity. For example, the same argument the author uses in Chapter 14 about there being at least as many bacterial species as beetle species would apply equally to bacteriophage diversity relative to bacterial diversity.

The third section of the book covers modern approaches to study microbial communities, including identification of uncultivated organisms, sequence-based surveys, molecular fingerprinting methods, stable isotope probing, and fluorescent in situ hybridization. The five chapters in this section include detailed discussions of case studies from the literature to illustrate the application of these methods. These “Study and Analysis” sections invite students to refer to the original articles while reading the chapter. Questions related to these studies appear at the end of the chapters. While this is a common approach for an advanced undergraduate course, and case studies are often summarized in textbooks, it is unique to have detailed analysis of literature integrated into the textbook itself. The studies chosen for these exercises may or may not be true “classics,” but are certainly interesting enough that they should remain relevant for some time.

The concluding section has two short chapters: the first touches a bit more on genomic and metagenomic analyses, and the second on the origins of life. This material seems a little out of place to me: the genomic and metagenomics analysis could easily fit in the preceding section, and the origins of life would fit nicely in the introduction section. On the other hand, coming full circle is a poetic way of ending a book.

*Principles of Microbial Diversity* is written in a clear and conversational tone. The style and content seems to be shaped by the author’s own classroom teaching experiences and passion for the subject, which at times gives it a personal touch. However, the more technical aspects are generally conveyed in a factual style typical of textbooks, and the occasional editorial comments were not distracting. The pages are populated with numerous useful figures and attractive color photographs. The chapters are short and extremely readable. Each chapter ends with a list of “Questions for Thought,” some of which are very creative, open-ended, and challenging (for example, “Are viruses alive?” and “Can you draw a tree with deep branches that are not primitive, and with primitive branches that are not deep?”). One gets the impression that the author is an effective and engaging teacher.

In summary, *Principles of Microbial Diversity* is unique among undergraduate textbooks in its focus, and should fill a niche held by few other textbooks. It presents a modern framework for understanding microbial diversity, efficiently surveys many of the microbial taxa, and presents some of the important tools used to study microbial diversity in the context of real case studies from the recent literature. The author clearly had to make some difficult content choices to keep this book compact, affordable, and accessible. Your favorite microbe may not have made it into this textbook (especially if you’re a mycologist or virologist), but you will probably agree that it strikes a good balance between detail and clarity. *Principles of Microbial Diversity* succeeds in imparting a sense of the vast evolutionary, physiological, and ecological diversity of the microbial world, along with the essentials of the major phyla and the tools of the trade. It reads well and the study questions encourage critical thinking. I would expect this book to serve as an excellent text for a course in microbial diversity, or as a supplement for a broader course in microbiology.

David Lipson
Department of Biology
San Diego State University
San Diego, Calif.
ASM Meetings Calendar

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

17–21 September 2015.
ICAA/ICC Meeting.
San Diego, Calif.

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/

13–17 April 2016.
13th ASM Conference on Candida and Candidiasis.
Seattle, Wash.
WWW, http://conferences.asm.org/

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Noncoding DNA and Bacterial Evolution
by S. Marvin Friedman

The rapid pace of bacterial evolution has long been thought to derive from horizontal gene transfer (HGT) as well as mutations and gene duplications. HGT allows a bacterial strain to acquire novel, useful traits through acquisition of DNA from nonparental strains. Far less attention has been given to HGT of noncoding DNA that may contain regulatory elements such as promoters. Isn’t the transfer of regulatory elements at least as important as that of coding genetic sequences? Oren and coworkers have investigated the extent of noncoding DNA exchange, or “regulatory switching,” and its phenotypic consequences.

The highly conserved core genes that encode essential “housekeeping” functions were not expected to undergo regulatory switching. However, comparing 1,479 core genes across 46 Escherichia coli genomes revealed that the ancestry of the upstream regulatory regions of 13% of them appeared to differ from that of their coding region. At least one of these regulatory regions was transferred across species, in this case from Enterobacter to E. coli. Further, two distinct regulatory variants were identifiable for 11% of the core genes—a remarkable amount of regulatory switching among a crowd known for its conservative traits! The authors dubbed this process “horizontal regulatory transfer” (HRT).

If the incidence of HRT in core genes is ~11%, wouldn’t we expect an even higher incidence in accessory genes, those not involved in essential functions, which are generally less conserved? Surprisingly, this was not the case, perhaps because the likely mechanism involves homologous recombination, and thus requires short stretches of sequence identity between donor and recipient. By this thinking, the most fertile grounds for HRT are the most conserved genomic regions.

Of interest, they found that HRT took place among all functional categories, including global regulators. For instance, 32% of the promoters in their data set were acquired horizontally and independently of the genes they regulate. How might this impact the gene transcription start sites (TSSs)? Comparing across 40 E. coli strains they found that these acquisitions increased the mean divergence among TSS positions fivefold.

Does regulatory switching alter the transcriptional response? RNA-seq was used to compare expression patterns of a set of orthologous genes in two strains of E. coli, one a gastric commensal and the other a uropathogenic strain. Gene expression levels were measured under different growth conditions to identify genes whose expression changed in response to the environment. They found 266 such genes when the strains were grown in defined minimal media, and 219 when they were grown in pooled human urine. Moreover, these environmentally responsive genes were enriched threefold for genes showing HRT. Of the switched genes that showed expression divergence, 45% did so under only one of the growth conditions, thus highlighting the importance of this phenomenon in allowing the bugs to respond to environmental cues.

Uropathogenic E. coli has to deal with oxidative stress generated by the host immune cells. Interestingly, the gene whose expression varied the most during growth in urine, metE, is known for high sensitivity of its gene product, methionine synthase, to oxidation. Might regulatory switching confer a fitness advantage under certain stresses, in this case, oxidation? To test this, a strain was constructed that was identical to the parental uropathogenic strain, except that the parental regulatory allele was replaced with the allele found in commensal strains. This new strain grew normally on minimal media lacking methionine, but under oxidative stress it showed a marked growth defect relative to the parental strain. Thus it appears that a single HRT event—with no change to the gene’s coding region—made a pathogenic strain more resilient, elucidating the selective pressure behind this particular regulatory switch.

When they extended their analysis to nine additional taxa, representing a broad range of characteristics including being gram-positive, they found that all clades exhibited HRT in core genes, the extent varying from 0.5% in the pathogen Chlamydia trachomatis to 15% in the highly recombinogenic pathogen Neisseria meningitidis. They envision that HRT allows a bug to explore mutational space while maintaining the coding regions of essential genes. The extent of regulatory switching observed among core genes affords them a larger role than previously suspected in phenotypic adaptation and diversification.

S. Marvin Friedman is Professor Emeritus, Department of Biological Sciences, Hunter College of CUNY, New York City
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