Current Topics

Genomic Analyses Could Lead to “Field Guide to Microbes”

The swell of enthusiasm for analyzing microbial genomes continues, with keen interest in doing more and more genomes in smaller analytic formats at lower costs. Even while greater numbers of microbiologists jump into this fray, some continue to fret over what to make of these expanding findings, sharing thoughts and insights during several sessions, including the symposium “Microbial Single-Cell Genomics” and the colloquium “The $1 Bacterial Genome,” convened during the 109th ASM General Meeting, held last May in Philadelphia, Pa.

“Single-cell genomics is a reality,” says symposium participant Paul Blainey of Stanford University in Stanford, Calif. He described an approach that depends on microfluidic devices and other comparably scaled manipulative procedures for plucking single bacterial cells, including from human anatomic sites such as the skin and oral cavity, for genomic analysis. “You need 1 pg or less of DNA to carry out a sequencing run, even though manufacturers recommend samples containing 1,000 times more material,” he says.

Microbial genomic sequencing is not merely driving miniaturization and other innovative technology-development projects. It is also leading some experts in the field to ponder new means for collating and analyzing the volumes of sequencing data being produced, and also to identify which segments of the microbial world are underanalyzed and thus still poorly appreciated in the new genomics-centric scheme, according to colloquium participant Jonathan Eisen of the University of California, Davis, Genome Center.

For instance, a genomic encyclopedia project sponsored by the Department of Energy lays out which phyla include species whose genomic sequences are already analyzed, which are not, and which ones are “sparsely populated,” Eisen points out. Such collections can provide a valuable “scaffold” for sorting genomic data and also for “annotating predicted functions,” he says, suggesting such efforts could lead to a “field guide to microbiology.”

“A massive amount of diversity is untouched by genomic sequencing,” Eisen continues. On the microbial side, viral and eukaryotic microorganisms are both “poorly sampled.” Moreover, he says, “We need to fill in the tree of life in terms of experiments, not just sequencing... ‘Meta-data rocks’ when it’s about the microorganism and its known physiology. I didn’t realize at first how fundamentally important this would be.”

Amid enthusiasm for genomic sequencing, including at the single-cell level, and other analytic approaches to enrich and extend sequencing come some startling predictions, including that sequencing will bring about the downfall of microarray analyses, according to another colloquium participant, Julian Parkhill of the Sanger Institute in Cambridge, United Kingdom. “Highly dynamic and accurate” sequence-based analyses of single-stranded RNA molecules “eventually will replace microarrays” to measure gene expression, he asserts. “They’ll be gone... and we’ll be doing phenotyping at the rate we do genotyping in the very near future.”

Moreover, Parkhill says, sequencing can be used as an “assay” to track the geographic spread of specific bacterial strains responsible for hospital and community outbreaks, including those involving methicillin-resistant *Staphylococcus aureus* (MRSA). With that approach, a particular MRSA outbreak in England was “traced back to a nurse from Thailand. This is really exciting stuff.”

Others working in this field also anticipate genome-based analyses being harnessed for similarly pragmatic purposes. For instance, sentinel genomic analysis might be used to “track epidemics, like a weather map,” says colloquium participant George Church of Harvard University Medical School in Boston, Mass. “You could study the microorganisms [pathogens] themselves, or host immune responses to them.”

“Genomic analysis could be helpful for following foodborne disease outbreaks,” adds Paul Keim of Northern Arizona University in Flagstaff. He draws that conclusion from a study that entailed applying genomic analysis to determine how *Bacillus anthracis* strains, which associate with bison and cattle herds, came to be distributed throughout regions of North America. *B. anthracis* apparently “went from Canada south, and from bison to cattle, instead of from south to north,” he says. “It’s just the opposite of what we’d believed before.”

Meanwhile, some microbiologists are touting the advantages of applying genomic analyses to single bacterial cells. “Why do single-cell analysis on something you can culture?” asks...
symposium participant Roger Lasken of the J. Craig Venter Institute in San Diego, Calif. One reason is that “culturing modifies genetics, and virulence factors can be lost,” he says. Plus, he adds, “It’s an exciting way to study pathogens. You can look at wounds [and recover] single cells.”

Single-cell analyses require scrupulous care in preparing reagents and maintaining quality controls at several different levels, Lasken continues. For one thing, DNA contaminants are everywhere. Another challenge is finding appropriate methods and conditions to lyse particular bacterial cells to ensure that they release their DNA molecules. Single-cell analysis can prove useful in searching for new species as part of a microbiome project, he points out. “One way to get rid of human DNA is to [analyze] a single cell instead of using a skin swab.”

Microfluidic devices also help to overcome the challenge of extraneous DNA contaminants when doing single-cell genomic analyses, adds Blainey of Stanford University. “Inside the device, there’s no pipetting,” he says. Another advantage is its “precise control, eliminating sample-borne contaminants by reducing the volumes used [and] by bringing thoroughly washed cells into the reaction chamber.”

Expanding such plans and uses of genomic analysis is expected to accelerate as costs come down, according to Church of Harvard Medical School. “I think we can do better than $1 per genome, and maybe get down to $0.01,” he says. “We’ve gone 4 logs in 4 years, and I predict we’ll go another 2 logs in the next 2 years.” Which of several technologies will prove key in realizing those log-order cost savings is uncertain, but nanopore-based and electron microscopy-based contenders are both “making progress,” he notes. Moreover, at least 15 companies are in the running with these technologies, providing improvements of one sort or another in terms of advances, cost and time savings, portability, or other useful features.

**Jeffrey L. Fox**

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### Antifungal Compounds from Seaweed Show Antimalarial Potential

Several natural compounds made by and that protect *Callophycus serratus*, a red seaweed, against marine fungi also inhibit the malarial parasite *Plasmodium* but not *Candida albicans*, a yeast that is an opportunistic pathogen of humans, according to biologist Julia Kubanek of the Georgia Institute of Technology (GIT), Atlanta, and her collaborators. Although the link between antifungal and antimalarial activity is not understood, these dual-acting natural compounds might work via a common mechanism, she says. “Learning how other species avoid diseases may provide us with tools we can use to avoid or treat our own diseases.”

Kubanek and her collaborators are analyzing natural compounds from more than 800 marine organisms that were collected near the Fiji Islands. Recently, they came to focus on the red seaweed *C. serratus*, which produces at least 28 distinct chemical compounds to block growth of the common marine fungal pathogen *Lin- dra thalassiae*. More than half—18—of the antifungal compounds are bromophycolides, while the other 10 are callophycoic acids. The bromophycolides and callophycoic acids form the largest group of algal antifungal compounds reported to date.

These antifungal compounds are found in discrete patches along the surface of the seaweed, and at concentrations sufficient to inhibit fungi, according to Kubanek’s colleague Facundo Fernandez at GIT. He and members of his lab group are using desorption electrospray ionization mass spectrometry (DESI-MS) to analyze features of the surface chemistry of intact organisms. “We hypothesize that the patches are wound sites where the compounds are made or leak out to create a barrier against fungal invasion,” Kubanek says. Details appeared online in the *Proceedings of the National Academy of Sciences* on May 5, 2009.

Although none of the antifungal...
compounds kill *C. albicans* cells, three of the bromophycolides have antimalarial activity, possibly through a common mechanism of action such as interfering with cell cycle functions, according to Kubanek. Other bromophycolides show modest anti-HIV and anticancer activity, she points out.

Kubanek finds it surprising that this seaweed produces so many—namely, 28—structurally related antifungal chemicals. “These compounds may have other functions that we have not yet discovered,” such as staving off predators or preventing fouling by spores and larvae of other microorganisms, she says. Alternatively, enzymes with low specificity may generate diverse bromophycolides and callophycoic acids. “Evolution is not perfect, and some enzymes do not synthesize the exact molecule every time.”

From seaweed samples belonging to the same species, a population nicknamed “bushy” makes only bromophycolides, while another population makes only callophycoic acids, according to Kubanek. Findings of this sort could provide insights into seaweed and microbial ecology, she says.

The surface-associated bromophycolides and callophycoic acids are secondary metabolites that govern biological interactions among organisms sharing an ecosystem, according to Paul Jensen, a microbiologist at the Scripps Institute of Oceanography in La Jolla, California. These secondary metabolites have “been the black box of microbial chemical ecology,” he says. Now Kubanek and her collaborators “cracked open this box with the application of DESI-MS to directly determine the location and concentration of specific secondary metabolites on algal surfaces.” Not only do the findings confirm that marine algae use antimicrobial defenses, he adds, but DESI-MS also provides an exciting new tool to explore questions in microbial chemical ecology.

**Bill on Farm Use of Nontherapeutic Antibiotics Inches Forward**

Despite steadfast opposition as well as political uncertainties within the U.S. Congress, there were renewed signs last July of forward momentum for H.R. 1549, the Preservation of Antibiotics for Medical Treatment Act (PAMTA). Moreover, top officials of the Food and Drug Administration (FDA) signaled, in part through their “public health approach” to agency business, that further restricting nontherapeutic agricultural uses of antibiotics is warranted. This new momentum was evident during a hearing before the U.S. House of Representatives Rules Committee, which was convened in July by chairwoman Representative Louise Slaughter (D-N.Y.), the only microbiologist in the U.S. Congress, who is keenly interested in public health issues—enough so to bring such matters before the Rules Committee, which typically focuses on parliamentary procedures.

“We cannot afford for our medicines to become obsolete,” Slaughter says. “As a microbiologist, I cannot stress the urgency of this problem enough.” In particular, seven classes of antibiotics—penicillin, tetracyclines, macrolides, lincosamides, streptogramins, aminoglycosides, and sulfonamides—that FDA deems to be “highly” or “critically” important in human medicine continue to be used for nonmedical purposes in agriculture, she points out.

“Despite their importance in human medicine, these drugs are added to animal feed as growth promotants and for routine disease prevention,” Slaughter continues. “Although the FDA could withdraw its approval for these antibiotics, its record of reviewing currently approved drugs under existing procedures indicates that it would take nearly a century to get...
these medically important antibiotics out of the feed given to food animals.” PAMTA seeks to phase out their non-medical use much more quickly.

“FDA supports the idea of H.R. 1549 to phase out growth promotion/feed efficiency uses of antimicrobials in animals,” says Joshua Sharfstein, Principal Deputy Commissioner at FDA. “Protecting public health requires the judicious use in animal agriculture of those antimicrobials of importance in human medicine. To avoid unnecessary development of resistance under conditions of constant exposure — growth promotion/feed efficiency — to antibiotics, the use of antimicrobials should be limited to those situations where human and animal health are protected.” However, he notes, “Legislation should permit the judicious use of antimicrobials in animals for prevention and control.”

“The present system of producing food animals in the United States is not sustainable and presents an unacceptable level of risk to public health, damage to the environment, as well as unnecessary harm to the animals we raise for food,” says Robert P. Martin, a senior officer at The Pew Environment Group in Washington, D.C., who cited a 2008 report from the Pew Commission on Industrial Farm Animal Production when he testified before the House Rules Committee. “The Commission was so concerned about the indiscriminate use of antibiotics in food animal production, and the potential threat to public health, that five of those recommendations deal with antibiotic use, including a call for the end of the nontherapeutic use of antibiotics in food animal production and strict definitions for their use.

“It is important to note that the Pew Commission never advocated ending all antibiotic use in food animal production,” Martin continues. “Such a recommendation would be irresponsible. We did seek to maintain the effectiveness of antibiotics to treat sick animals by limiting the routine use. The increase in bacterial antibiotic resistance, and the inappropriate use in food animal production, is a serious — if silent — threat to our public health.”

“Regular antibiotic use in food animal production is an unnecessary public health risk and a crutch for improper animal husbandry practices,” says microbiologist Lance Price, director of the Center for Metagenomics and Human Health at the Translational Genomics Research Institute in Phoenix, Ariz., who also testified during the July hearing. “Until there is legislation to prevent unnecessary use of antibiotics, then most producers will continue to use antibiotics to patch their outdated practices. [PAMTA] is a solid first step towards curbing unnecessary antibiotic use in food animal production.”

Jeffrey L. Fox

New Extremes for Earth Habitats Supporting Microbial Species

Mat-like photoautotrophic microbial communities thrive in volcanic vents, or fumaroles, at 6,051 meters above sea level, making this the highest known natural microbial habitat, according to microbial ecologist Steve Schmidt from the University of Colorado, Boulder, and his collaborators. Meanwhile, drawn from a nearly opposite extreme habitat, the ultrasmall brown-purple microorganism Hermimonas glaciei sp. nov. recently earned the distinction of being the “only species isolated from Greenland ice that has been fully characterized and validly named,” according to Jennifer Loveland-Curtze of Pennsylvania State University in State College and her collaborators.

Dormant cells of H. glaciei were extracted from ice cores that were drilled from 3,042 meters below the surface of the Greenland ice sheet 15 years ago and stored at the National Ice Core Laboratory. After being held anaerobically for many months in liquid culture and then plated on tryptic soy agar at low temperature, one of the core samples recently yielded viable ultra-microbacteria, whose cells are 50-fold smaller than those of Escherichia coli. The brown-purple bacteria had been dormant for at least 120,000 years, according to Loveland-Curtze and her collaborators. Details of their findings are published

Minicells Effectively Deliver Antisense and Cytotoxic Agents to Tumor Cells

Bacterially derived minicells provide a vehicle for delivering anticancer agents, including small interfering RNA (siRNA) duplexes to overcome drug resistance and, separately, cytotoxic drugs to destroy malignant cells directly, thereby reducing overall levels of drugs administered to lower the risk of systemic toxicities, according to Himanshu Brahmbhatt of EnGeneIC Pty Ltd. in Sydney, New South Wales, Australia, and collaborators at several nearby institutions as well as at Cold Spring Harbor Laboratory in Cold Spring Harbor, New York. Part of the treatment strategy entails affixing antibodies to the surface of minicells to target them to tumor cells. The minicells, which derive from a minCDC deletion mutant of Salmonella enterica serovar Typhimurium, produced “no adverse side effects or deaths” in the mice that were treated during these preclinical experiments. Details appear in the June issue of Nature Biotechnology.

Ultra-microbacteria should not be confused with nanobacteria, Loveland-Curtze says. “The existence of nanobacteria is questioned by many scientists, whereas ultra-microbacteria...predominate in many environments, with some species being fully characterized and officially validated.”

Meanwhile, Schmidt from the University of Colorado and his collaborators collected specimens from fumarolic and nonfumarolic soils on Socompa volcano, located between Argentina and Chile, in February 2008, when temperatures sometimes fluctuated from $-10$ to $+50^\circ C$ within one day. The nonfumarolic soils, which contained mainly actinobacteria and fungi, were extremely dry and almost devoid of organic nutrients. In contrast, the fumarolic soils had adequate moisture and nutrients, supporting a broad assortment of phototrophs, according to Schmidt.

Details appear in Applied and Environmental Microbiology (75:735–747, 2009)

Do phototrophs and other microorganisms hitchhike to the tops of volcanoes? “The bacteria that are in the nonfumarolic soils are mostly of the Spartinobacteria group of the Verrucomicrobia,” Schmidt says. “They probably...blew in on winds as dormant spores attached to dust particles. The phototrophs that live near the fumaroles are very diverse, encompassing many groups of algae and cyanobacteria. All these phototrophs are intensely pigmented—almost black rather than green—indicating their biggest stressor is the intense UV radiation at over 6,000 meters.” Little else is known, he adds, about “where these phototrophs are coming from and what adaptations are needed for them to survive in such a harsh environment.”

“Recently geologists recognized the all-pervasiveness of microbial communities...on Earth,” says geologist David Krinsley of the University of Oregon, Eugene. Fumarolic soils on Socompa volcano support “diverse microorganisms,” whereas nonfumarolic soils, which are poor in nutrients but otherwise comparable, support relatively simpler and fewer microorganisms. “Thus nutrients are important and temperatures and air pressures less so, [suggesting] that the

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**Exxon Mobil and Synthetic Genomics Join in Effort To Make Fuels from Algae**

Exxon Mobil of Irving, Tex., and Synthetic Genomics, Inc. (SGI) of La Jolla, Calif., in July formed an alliance to harness photosynthetic algae for the purpose of producing fuels. The $600-million deal “could be a meaningful part of the solution in the future if our efforts result in an economically viable, low net carbon-emission transportation fuel,” says Emil Jacobs, a vice president at ExxonMobil Research and Engineering Company. “This agreement represents a comprehensive, long-term research and development exploration into the most efficient and cost-effective organisms and methods to produce next-generation algal biofuel,” adds J. Craig Venter, founder of SGI. “We are confident that the combination of our respective expertise in science, research, engineering, and scale-up should unlock the power of algae as biological energy producers in methods and scale not previously explored.” Scientists at SGI recently engineered algal strains to produce lipids via a continuous process.
planet Mars could support microbial communities if the necessary nutrients were present,” he says.

“The work of Loveland-Curtze and her collaborators demonstrates the patience required to isolate microorganisms that colonize low temperature environments,” says Jill Mikucki, a geomicrobiologist from Dartmouth College in Hanover, N. H. who specializes in subglacial ecosystems. “Their effort provides the research community with a new cultured representative from icy environments. Understanding the physiology and genomic machinery of this deep-ice isolate will improve our perspective on icy life in general and will assist with formulating the next generation of questions regarding microbial survival in ice.” Both of these research examples are “highly relevant to the search for evidence of life on Mars,” adds Christopher McKay of the National Aeronautics and Space Administration Ames Research Center in Mountain View, Calif.

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Degradative Thermophile Is Promising Basic Step on Path from Biomass to Fuels

Thermophilic bacteria can degrade woody plant biomass from poplar and switchgrass, without any high-temperature chemical treatment—a key step toward developing renewable petroleum substitutes, according to Michael Adams of the University of Georgia, Athens and the Oak Ridge National Laboratory in Oak Ridge, Tenn., and his collaborators there and at the National Renewable Energy Laboratory (NREL) in Golden, Colo. Details are published in the July Applied and Environmental Microbiology (75:4762–4769).

The challenge of deconstructing cellulose and lignocellulose lies in their physical and chemical properties, which make these materials “recalcitrant” to the degradative efforts of microorganisms and insects such as termites. For instance, cellulose’s crystalline structure renders it insoluble, a deterrent to the degradative effects of enzymes that work in aqueous solutions. Further, even chemically treated lignocellulose requires 100-fold more enzyme to be degraded into component sugars than does starch, according to experts at NREL.

Nonetheless, Anaerocellum thermophilum DSM 6725, which is active at 75°C, degrades switchgrass, metabolizing both crystalline cellulose and xylan. Although other microorganisms metabolize cellulose and xylan, the authors describe their report as the first to document a microorganism degrading switchgrass. “The organism grew just as well on the insoluble biomass after we had removed any soluble material,” Adams says. It “also grew on what is termed ‘spent’ biomass, which is the insoluble biomass left over after the organism has already grown to a high cell density.”

To more fully degrade these recalcitrant materials, the researchers subject them to successive inoculation and culturing, an approach that helps to overcome a stalling out that occurs with diminishing returns in terms of completing the degradative process, according to Adams. This plateau effect might be due to quorum sensing, he says. In any case, after the third round of culture, A. thermophilum had converted more than 60% of the original switchgrass into soluble material.

Adams and his collaborators are identifying the enzymes and other proteins that partake in this degradative process. “The organism appears to use free enzymes, and does not produce a so-called cellulosome, the large extracellular multiprotein complex that degrades cellulose in some other anaerobic bacteria,” he says. Meanwhile, a team at the Department of Energy Joint Genome Institute (JGI) in Walnut Creek, Calif., determined the genomic sequence of A. thermophilum.

Those enzyme-driven degradative steps are part of the early phase of an anticipated process for making petroleum substitutes. The breakdown products from biomass, including hydrogen, acetate, lactate, glucose, and cellobiose need to be converted to

H1N1 Influenza, Like 1918 Flu, Is Especially Damaging to Lungs

The new pandemic H1N1 influenza viral strain that is now circulating causes more lung damage than does a seasonal influenza strain in several tested species, including mice, ferrets, and macaques, but does not cause disease symptoms in pigs that it infects, according to Yoshihiro Kawaoka of the University of Wisconsin in Madison and his collaborators (see p. 405). Further, this H1N1 flu strain appears to be sensitive to antiviral drugs, including Tamiflu, when tested in vitro. Based on the finding that antibodies from flu patients born before 1920 can recognize the new strain, the current recirculating H1N1 strain appears to be closely related to the influenza virus that was responsible for the 1918 pandemic. However, individuals born after 1920 apparently do not harbor antibodies that recognize the new strains. Details appear in the 13 July online issue of Nature [DOI: 10.1038/nature08260].
something else before they could serve as a substitute for gasoline. Toward that end, the research is “positive . . . although more incremental than breakthrough,” says James D. McMillan of NREL, who was not involved in the research. For example, the genes or genetic pathways embodied in *A. thermophilum* might be used to develop strains that could not only degrade biomass but also produce a substitute fuel such as ethanol or some other alcohol, he says.

Another shortcoming with *A. thermophilum* at this point is that it is not effective unless the biomass it digests is first milled into particles of less than 1 mm in diameter, which is “somewhat expensive,” McMillan notes. Moreover, these experiments were “done on extremely dilute suspensions of particles,” containing 0.5% weight per volume, whereas a commercial process could be expected to involve suspensions that are at least 20 times more concentrated.

This research remains in the “discovery” phase, and has not reached a “proof of concept of practical applicability,” McMillan says. Nonetheless, it could point the way to producing commercially viable fuels from biomass crops, including poplar trees and switchgrass that could be grown on marginal lands that are unsuited for food crops.

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