Current Topics

Natural Antibiotics — Small Molecules with Large Repertoires

“Our magic bullets have far more peaceful uses in the wild,” says Marvin Whiteley at the University of Texas at Austin, referring to natural substances with antimicrobial activities. “Many known antibiotics are gene modulators, not weapons, requiring us to challenge our preconceptions about their ecological roles.” The “view of antibiotics as therapeutic weapons is so strongly entrenched in our experience that we’ve ignored the more important functions these ubiquitous small molecules have in nature,” adds Julian Davies from the University of British Columbia in Vancouver, Canada. They and other microbiologists are reappraising what such molecules do in natural settings, where their signaling activities appear more important than killing their microbial neighbors.

“Whiteley’s research supports this concept and makes a very compelling argument for taking a broader view of the roles low-molecular-weight compounds play in nature,” continues Davies, who pioneered the view that the compounds we use in high concentrations as antibiotics exhibit very different biochemical properties at subinhibitory levels. “Bacteria are naturally gregarious, usually crowding together in huge, mixed-population communities that are both highly competitive and highly communicative. It seems reasonable that the small-molecule-producing microbes living mainly in soil and never meeting up with human pathogens are using what we call antibiotics for something else entirely and, although we still need more experimental proof, an important part of that something else appears to be communication.”

However, the notion that some bacterially generated molecules are strictly dedicated to communication is also unlikely, Whiteley asserts. “What we seem to have are poisons that signal, and signals that poison—a killing-signaling duality that could have important clinical applications. But only when we fully understand the nuances of their languages will we be able to decipher bacterial messages and manipulate them to our advantage.”

Unraveling these biochemical languages with their “numerous dialects and words that have multiple meanings is a Herculean task,” Davies says. “How can one even begin to comprehend a language spoken by correspondents that are largely unknown? And how can the correspondents be identified when 99% of them can’t be grown in the solitary confinement of a lab?”

Nonetheless, bacterial communication, or quorum sensing (QS), is now a matter of intense research interest. “Bacteria chatter continuously, and their words are chemical,” says Bonnie Bassler at Princeton University in Princeton, N.J. Her studies of Vibrio harveyi, a marine bacterium that lights up when quorum numbers of signal molecules are reached, provides a good example of this phenomenon.

“There is much more to QS than just direct information trafficking,” Whiteley says. “Take Pseudomonas aeruginosa, which kills competing bacteria and hijacks their iron stores using its Pseudomonas quinolone signal (PQS) as a weapon.” The ability to bind iron undoubtedly confers an additional advantage on this already formidable opportunist. PQS not only signals for higher expression of virulence genes, including those involved in iron homeostasis, it also appears to sequester iron, thereby increasing the bacterium’s overall competitiveness, he points out. Furthermore, PQS moves between cells in outer membrane vesicles (OMVs), and P. aeruginosa cells depend on PQS synthesis to form OMVs. “Thus, PQS is not only a potent communication signal, it physically assists in the delivery of itself and other vesicular cargo, probably by altering the properties of bacterial cell membranes,” he says.

Pseudomonads also secrete secondary metabolites called phenazines that are structurally related to PQS. Phenazines have long been studied as redox-active poisons, generating toxic by-products such as oxygen and hydrogen peroxide in anaerobic environments. “However, scratch the surface of a biofilm and there’s an anaerobic world, similar in some important respects to conditions on Earth billions of years ago,” says Dianne Newman of the Massachusetts Institute of Technology in Cambridge, Mass. Some microorganisms produce acylated phenazine compounds in subtoxic concentrations under anaerobic conditions. For example, they serve as important electron carriers in the membranes of some methanogenic archaea. Phenazines likely arose in an oxygen-free world, and their “antibiotic” effect could reflect the geochemical conditions prevalent on Earth today, she says.

Phenazines also act as electron shuttles for iron (III), aid in iron (II) acqui-
sition, modulate intracellular redox homeostasis, and play a pivotal role in biofilm development, dramatically affecting the morphology of multicellular communities, says Newman. Phenazines are much more than antibiotics, she stresses. “They profoundly affect the producing organism metabolically and developmentally.”

“Having diffusible QS signals with far-reaching effects in distance and scope was undoubtedly as vital in a primordial world as it is today,” says Whiteley, whose recent thoughts on this subject are summarized in the May 2009 Trends in Microbiology. “Then, like now, bacteria liked to gather in high-density populations—a safety-in-numbers strategy.” As more species arose, the need to compete for metabolites and niches also grew. With time, QS molecules gained a wider role in community protection and nutrient scavenging—doing more than just interacting with regulatory proteins to change gene expression. However, the more peaceful activities of the small molecules that we simplistically call “antibiotics” are not being discovered as quickly as Davies would like. “There are not yet enough believers,” he contends.

Marcia Stone
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A Potpourri of Probing and Treating Biofilms of the Oral Cavity

Decades before biofilms were named, microbiologists studying bacteria in the oral cavity realized that complex microbial communities form plaque on tooth surfaces—perhaps the prototype for much of biofilm research today. Some of that oral cavity-focused biofilm research is leading to new products to combat plaque and bad breath, while other efforts are providing insights about how microorganisms behave within these complex oral communities. Here are some highlights from the 25th annual Biofilm Science & Technology (BST) meeting, convened at the Center for Biofilm Engineering (CBE) at Montana State University (MSU) in Bozeman last July.

More than 700 types of microorganisms grow in the mouth, many of them helping to form plaque along tooth surfaces. Within minutes after a professional hygienist removes plaque, however, it begins reforming. One candidate for impeding plaque is ficin, a cysteine protease derived from the sap of Ficus glabrata trees, says microbiologist Harsh Trivedi from Colgate-Palmolive Company in Piscataway, New Jersey, who spoke during BST. U. S. Food and Drug Administration officials long ago assigned ficin, which is used widely as a meat tenderizer, to the “generally regarded as safe” (GRAS) list—a status that lowers regulatory hurdles for other uses.

Ficin potently prevents biofilms from forming using an anti-adhesion assay as well as an extracted human teeth treated in flow chambers, Trivedi says. It also penetrates with up to 90% efficiency a three-species biofilm containing Actinomyces naeslundii, Streptococcus gordonii, and Streptococcus oralis, all early colonizers of dental surfaces. In other tests involving human volunteers, use of a ficin-containing toothpaste decreases Porphyromonas gingivalis and changes levels of Fusobacterium and Porphyromonas species in plaque specimens. Although brushing with a ficin-containing toothpaste reduces plaque by 45% initially, after 6 weeks that plaque reduction drops to only 12% compared to individuals who use ordinary toothpaste, making ficin’s commercial future uncertain, he says.

Another biofilm-related oral health issue involves bad breath. “No one wants bad breath or to have to smell bad breath,” says dentist Alessandra Agostinho of the University of Sao Paulo, Brazil, a visiting scientist at the CBE and another participant at the BST meeting. About half of adults in the United States suffer from persistent halitosis, which is considered a clinical disorder. One likely source of halitosis is anaerobic gram-negative bacteria in oral biofilms that produce...
volatile sulfur compounds (VSC), including hydrogen sulfide and methyl mercaptan, she says.

To develop more precise information about the source of halitosis, Agostinho scrapes biofilms off tongues of volunteers, applies them to hydroxyapatite-coated glass slides, and then measures VSC concentrations. Halitosis is considered clinically significant when VSC levels rise above 1,000 ppb; the lab-tested biofilms give average readings of 1,490 ppb. When such biofilms are treated with a commercial toothpaste, mouthwash, sodium triphosphate (STP), or decapinol, the latter substance reduces plate counts by 99%, whereas the mouthwash reduced them by about 80%, and STP by 50%, she reports.

Robert Palmer, a microbiologist at the National Institute of Dental and Craniofacial Research at the National Institutes of Health in Bethesda, Md., is focusing on metabolic interactions that favor coaggregation of biofilm communities. The earliest colonizers of plaque include *Streptococcus* and *Veillonella*, and coaggregation, based on recognition between such cells, is suspected to play a role in biofilm development.

Palmer grew *S. oralis* and *V. atypica* PK1910 individually or together. Both types of microbe grow better in combination than as monocultures, especially *V. atypica* PK1910, whose growth is spurred by lactic acid from *S. oralis*. When *V. atypica* PK1910 nears *S. oralis*, it signals the latter to express amylase, which degrades stored glycogen to lactic acid via glycolysis. “That’s the benefit *Veillonella* gains,” says Palmer. “Coaggregation is important in the formation of biofilm communities.”

Mechanical procedures can scrape biofilms off teeth, but making those biofilms either softer or more brittle could speed their removal from teeth or other surfaces, points out Eric Brindle, a mechanical engineering graduate student at MSU. He is systematically testing how treatments with a range of agents, including urea, dispersin B, chlorhexidine, and iron chloride, can change the viscoelastic properties of biofilms. The extracellular polymeric substance (EPS) that holds biofilms together allows them “to act like rubber bands that stretch and return to the original position,” he says. Treatments with urea or dispersin B, an enzyme that chips away at the EPS matrix, renders *Staphylococcus epidermis* biofilms dramatically more viscous, he finds. In contrast, chlorhexidine—an ingredient in contact lens solutions and mouthwash—and iron chloride makes them stiffer. “Biofilm mechanical properties can be manipulated in desirable ways,” he says.

Carol Potera

Microbiologists Aim To Develop High-Precision Gene Ontologies

Traditional microbial nomenclature sometimes leads to misunderstandings, confusion, and other problems for microbiologists when, for example, they encounter the same gene within the type II secretion system through one or a few of its 20 or more different names—some of them based merely “on the phenotype it has in their bacterium,” says Alan Collmer of Cornell University, Ithaca, N.Y. To address this issue more broadly, biologists launched the Gene Ontology (GO) project in 1998, focusing then on mouse, fruit fly, and yeast genes. However, microbiologists soon brought bacterial and other microbiological nomenclature challenges into this forum, and the fruits from some of their focused GO efforts can be found in the July issue of *Trends in Microbiology* (TiM).

“The gene ontology is a standardized set of terms, words, and phrases with well-defined meanings which the community has reviewed,” says Brett M. Tyler of the Virginia Bioinformatics Institute (VBI), part of the Virginia Polytechnic Institute and State
Members of Congress Urge Further Study before Relocating Ag Pathogen Lab

Citing a July report from the Government Accountability Office (GAO), Representatives Bart Stupak and John Dingell, both Democrats from Michigan, are recommending an independent safety assessment before the Department of Homeland Defense (DHS) builds its National Bio- and Agro-Defense Facility (NBAF) in Kansas or elsewhere on the U.S. mainland. The proposed facility, which is intended to replace containment labs on Plum Island that were run by the U.S. Department of Agriculture (USDA), is to be used for research on pathogens, including the virus responsible for foot-and-mouth disease (FMD). DHS “gave inadequate consideration to the risks of transferring foot-and-mouth and other highly-contagious diseases to the mainland,” Stupak says. “Moving contagious animal research to the heart of America’s livestock industry remains a foolish tempting of fate.”

FMD is “the most highly infectious animal disease known,” according to the GAO report, GAO-09-747, “Biological Research: Observations on DHS’s Analyses Concerning Whether FMD Research Can Be Done as Safely on the Mainland as on Plum Island.”

University in Blacksburg, Va. The terms represent specific biological processes, cellular components, and molecular functions, regardless of species. The annotations include an “evidence code” that tells what experiments support each annotation, thus giving biologists a more precise idea of what their colleagues mean.

Without the GO, genomics would face an even more difficult challenge to fulfill its promise of making gene comparisons between different species, Collmer says. “If somebody with another organism has made more progress in understanding the function of that gene, being able rapidly to connect with that function when analyzing your [microorganism’s] genome is very powerful, but you can’t do it based on the gene’s name. The gene ontology provides a consensus universal language that operates across all organisms.”

“We specifically ran into this problem in connection with pathogenesis,” Collmer continues. Very different microbes interact with their respective plant hosts in similar fashion, regardless of whether the microorganism interacting with a particular host is a bacterium, a fungus, a nematode, or an oomycete. To describe these processes, the Plant-Associated Microbe Gene Ontology Consortium compiled more than 800 terms to describe such microbe-host interactions, according to one of the articles in the July TiM.

These efforts are expected to help when it comes to defining functions of different genes from sequences derived from mixed microbial populations in environmental samples. For example, the Human Microbiome Project plans to sequence metagenomes of five sites from the human body to learn more about how such microbial communities affect human health (see p. 451).

These improved GO terms will help humans program and then understand more fully the data sets that play such a vital role in tracking genomic analyses. “I can write a program that says ‘tell me all the genes in all microbes that have such and such a function,’ and it will instantly give me the results,” Tyler of VBI says. “Imagine doing a search on Google,” he says, contrasting its fuzziness with the crispness of a search using carefully defined GO terms.

To further strengthen the GO, the Gene Ontology Reference Genome Project (www.geneontology.org/GO.refgenome.shtml) is developing reference annotations for 12 of the most important model organisms spanning the tree of life, from Escherichia coli to Arabidopsis and the zebrafish. That focused exercise “will make it far easier to automatically annotate new genomes,” says Michelle Gwinn-Giglio of the University of Maryland School of Medicine in Baltimore.

GO is also being used extensively in microarray expression analysis and genome-wide association studies. The GO annotation repository, where GO annotation datasets are stored, contains hundreds of thousands of annotations for thousands of species. For microbiology, as well as biology generally, it is a powerful new tool. “I like to think of it as a universal biological language,” says Gwinn-Giglio. “You could compare it to the Rosetta stone . . . but maybe that’s a little over the top.”

David Holzman
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Many Contingencies To Face in Readying H1N1 Influenza Vaccines

Public health officials, vaccine producers, and other experts with responsibilities for countering influenza outbreaks are scrambling to develop, evaluate, produce, and deploy vaccines to protect against both seasonal and the new H1N1 pandemic flu viruses. With so much at stake and many uncertainties, they also are reviewing a range of contingencies, even while acknowledging that circumstances will likely eliminate some of those contingencies and give rise to
Recent Malaria Findings Include Evidence of Artemesinin Resistance

Here are some recent highlights from research on malaria:

- The parasite responsible for malaria, *Plasmodium falciparum*, shows reduced susceptibility to artesunate, the first-line artesminin-based combination therapy that is being used in western Cambodia, as compared with northwestern Thailand, according to Arjen Dondorp at the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, and collaborators there and at other institutions, including in Cambodia, Switzerland, and the United Kingdom. Resistance is characterized by slow parasite clearance in vivo without corresponding reductions in conventional in vitro susceptibility testing, they report in the July 30 *New England Journal of Medicine* (NEJM).

- Volunteers inoculated with radiation-attenuated *P. falciparum* sporozoites by means of more than 1,000 infective mosquito bites were “protected against a malaria challenge” and experienced “no serious adverse events,” according to Robert Sauerwein at Radboud University Nijmegen Medical Center in Nijmegen, the Netherlands, and his collaborators there and in France and Singapore. Their findings also are published in the July 30 *NEJM*.

- Malaria apparently jumped from chimpanzees to humans via mosquitoes, a conclusion based on analysis of eight new isolates of *P. reichenowi* from wild and wild-born captive chimpanzees in Cameroon and Ivory Coast, according to Francisco Ayala at the University of California, Irvine, and his collaborators. All extant *P. falciparum* populations originated from *P. reichenowi*, likely by a single host transfer as early as 2–3 million years ago or as recently as 10,000 years ago, according to their report in the online August 3 *Proceedings of the National Academy of Sciences*.

others. Key uncertainties as of late August included questions as to whether the H1N1 virus would better adapt to humans and become more virulent, how to overcome inefficiencies in vaccine productivity, whether to approve adjuvant use, what strategy to follow in using diagnostic procedures, some of which performed poorly during earlier phases of this pandemic (*Microbe*, September 2009, p. 405.)

Although the novel H1N1 flu virus continued to cause outbreaks in the Northern Hemisphere throughout the summer, it appears not to be “fully adapted” to humans, according to Nancy Cox of the Centers for Disease Control and Prevention (CDC) in Atlanta, Ga. During an advisory meeting convened in July by the Food and Drug Administration (FDA) Center for Biologics Evaluation and Research, she described efforts to analyze hundreds of flu virus isolates, including through genomic sequencing and via controlled studies with animals. One key finding, for instance, is that the H1N1 virus does not transmit between ferrets as efficiently as do seasonal flu isolates, she reports. This experimental result is consistent with epidemiologic patterns seen in human outbreaks.

Moreover, thus far, H1N1 isolates remain “very homogenous,” and there is little or no evidence that H1N1 is becoming more virulent as it continues to infect more and more people. Further, this virus tends to infect younger individuals while sparing older adults, particularly those older than 60, whose infections nonetheless can prove “dangerous,” Cox says. Consistent with those findings, many older adults apparently produce antibodies that cross-react with antigens of the circulating H1N1 virus, likely accounting for some of these age differences in immunity.

U.S. officials are anticipating having between 50 and 100 million doses of H1N1 flu vaccines available by October, and as many as 600 million doses by March of 2010. Meanwhile, the array of vaccines to protect against seasonal flu was expected to be plentiful and available sooner.

One big problem is that yields from the initial set of H1N1 vaccine strains were only about 30% for several of the killed-virus, injectable vaccines, according to Jerry Weir of FDA. “Manufacturers are looking for strains that are higher yielding,” he says. However, because the “reassortant strains” that are used for producing these vaccines uniformly “grow poorly,” he adds, expectations for boosting productivity were not high as of last July.

One exception to these vaccine-productivity problems is the MedImmune H1N1 flu vaccine, which is a live-attenuated vaccine, says Raburn Mal-lory of the Gaithersburg, Md.-based company, which is a subsidiary of AstraZeneca in London, U.K. This vaccine, which is delivered to individuals intranasally, is being produced with “good yields” that are “much like” those for the company’s live-attenuated seasonal flu vaccine, he says.

Poor productivity for several versions of the H1N1 injectable vaccine,
its anticipated scarcity from September through December when the flu season gains momentum in the Northern hemisphere, and the likelihood that its poor antigenicity will force a two-dose regimen for many recipients are encouraging manufacturers and public health officials to consider the use of adjuvants to improve efficacy and to stretch limited supplies.

According to some early tests, adjuvants improve the immunogenicity of the candidate vaccines. For example, the GSK H1N1 vaccine is “poorly immunogenic” on its own but “highly immunogenic in all age groups” when used with AS03, an adjuvant that contains α-tocopherol in an oil emulsion, according to Bruce Innes of the London-based company. Even with adjuvant, however, two doses are needed to confer a full response, he adds. Although this and other adjuvants are used widely in Europe, other experts note, there is little experience with them in the United States.

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Adjuvant use “is one of the hardest decisions before us,” says Robin Robinson of the Biomedical Advanced Research Development Authority (BARDA) in the Department of Health and Human Services (HHS) in Rockville, Md. “Ultimately, that decision will be made by the HHS Secretary or at the White House.” Experts who are members of several federal advisory committees also will have input before such decisions are made, adds Bruce Gellin, director of the HHS National Vaccine Program Office.

Amid these uncertainties, public health officials are building consensus as to what population groups will have priority for being vaccinated during the period when only limited quantities of the H1N1 vaccine will be available. Groups with high priority likely will include students and preschool children, pregnant women, health care workers, and older adults with medical impairments that would make them particularly vulnerable to flu infections. Vaccinating members belonging to some of these priority groups will amplify the impact of the vaccine, which is part of the strategy behind coping with limited supplies.

For instance, vaccinating pregnant women will help toward protecting infants who are less than 6 months old, while vaccinating school children should help in protecting their younger siblings against the virus.

“For routine cases of flu-like illnesses, there is little reason to apply diagnostic tests,” says John Modlin from Dartmouth-Hitchcock Medical Center in Lebanon, N.H., who chairs the FDA Vaccines and Related Biological Products Advisory Committee. However, when it comes to “assessing vaccine efficacy, there’s a need for improved diagnostics.” The use of diagnostic procedures to distinguish H1N1 from seasonal flu cases will be “limited,” but such testing remains “important as a surveillance tool,” agrees Robinson from BARDA, adding: “It’s a complex issue.”

Jeffrey L. Fox
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Tropospheric Microbes Are Surprisingly Diverse Yet Stable

Airborne microbial diversity is much greater than expected, albeit spare compared to that in the ocean and in the soil, according graduate student Robert M. Bowers, his advisor Noah Fierer, and their collaborators at the University of Colorado, Boulder, and elsewhere, who collected their data at Storm Peak lab in northwestern Colorado at an elevation of 3,200 meters. Moreover, the species of bacteria that they observe in the troposphere remain relatively stable over time, despite changing conditions. Further, ice nucleation—a precursor to precipitation—does not correlate with the local abundance of known ice-nucleating microbes. Details appear in the August Applied and Environmental Microbiology (75:5121–5130).

Roughly 100 species of bacteria and
equivalent numbers of fungal species were found in each of nine air samples from Storm Peak—far fewer than in urban or rural air samples, and fewer than in one sample of snow from there, which contained 800 species, Bowers says. Airborne microbial population density values range from $9.6 \times 10^5$ to $6.6 \times 10^6$ cells per m$^3$, with roughly equivalent numbers of bacteria and fungi. “These airborne microbes are likely to play some role in cloud formation, and can therefore be said to play a role in the global radiation budget,” he says.

“Gram-negative proteobacteria were the most commonly detected bacteria,” says Eoin Brodie of the Lawrence Berkeley Laboratory, who was not involved in the research. This dominance contrasts with previous findings, he points out. For example, in urban aerosols, spore-forming gram-positive bacteria such as the bacilli and actinomycetes tend to be dominant. He notes that because so few species in the air samples could be grown in culture, they were identified by rapid “pyrosequencing” of ribosomal genes.

“On average, about 40% of the organic carbon in the atmosphere is probably intact cells,” Fierer says. The stability of microbial populations within the troposphere, despite both changes in climate conditions and the source of the air, suggests that the atmosphere “is a tough place to live.” It takes a tough but stolid set of microbes to contend with high-intensity UV radiation, lack of food, and low moisture in that setting. Further, he asks, “What are these things doing up there? Do they have any influence on atmospheric conditions? There is recent data, and anecdotal evidence from Bob [Bowers] and others showing that some of the best nucleators are bacteria and fungal cells, so what percentage of nucleation is driven by cells?”

“Data such as these will help to constrain the biological contribution to atmospheric ice-nuclei populations so that numerical models can estimate the role of organisms in cloud processes,” says Paul DeMott of Colorado State University, Fort Collins. “It is intriguing because these particles represent a highly dynamic source. Climate may impact their abundance, and their abundance may impact climate by impacting cloud and precipitation processes.” He speculates that they might seed clouds via green plants.

“Proteobacteria have previously been implicated in ice nucleation,” Brodie says. However, the study findings do not support the idea of a relationship between abundance of ice-nucleating bacteria and numbers of ice nuclei. This discrepancy with earlier findings suggests to him that “either other, as yet unknown, bacteria are involved or that fungal spores or abiotic mechanisms of ice nucleation dominate the process at this site.”

David Holzman

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Researchers Report Microbiome Changes Could Explain Some Diseases

A global change in the esophageal microbiome might give rise to gastroesophageal reflux diseases (GERD), according to Zhiheng Pei at the New York University Langone Medical Center in New York, N.Y., and his collaborators. They find high concentrations of *Streptococcus* in the esophagus of healthy patients, but an altered microbiome dominated by gram-negative bacteria in patients with esophagitis and Barrett’s esophagus. “If changes in the bacterial population do indeed cause reflux, it may be possible to design new therapies with antibiotics, probiotic bacteria, or prebiotics,” he says. Details appear in the August 1 *Gastroenterology.*

Meanwhile, increases in interleukin-17-based cellular signaling appears to cause severe inflammation that can lead to colon cancer when enterotoxigenic *Bacteroides fragilis* bacteria (ETBF) colonize the gastrointestinal tract of mice, according to Cynthia Sears at Johns Hopkins University School of Medicine in Baltimore, Md., and her collaborators. Blocking that signaling prevents ETBF-induced inflammation and tumor formation, they report in the August *Nature Medicine.*