Current Topics

Bacterial Symbionts of Farming Ants Produce Cyclic Antimicrobials

Leaf-cutting, or attine, ants culture antibiotic-producing bacteria to defend their fungal gardens against predators. One of their selective antibiotics, an actinobacterially produced cyclic depsipeptide containing highly modified amino acids, was recently isolated and analyzed by Jon Clardy from the Harvard Medical School in Boston in collaboration with researchers there and at the University of Wisconsin in Madison. Their findings were reported online in the 29 March 2009 Nature Chemical Biology (doi: 10.1038/nchembio.159).

The newly characterized antimicrobial agent, named dentigerumycin in honor of Apterostigma dentigerum, the fungal-farming ant species that depend on its activity, “contains the unusual amino acids—piperazic acid, γ-hydroxypiperazic acid, β-hydroxy-leucine, and N-hydroxyalanine—and has the molecular formula C_{40}H_{67}N_{9}O_{13},” Clardy reports. Testing shows that dentigerumycin actively inhibits the fungal pathogen Escovopis while largely sparing the fungal cultivar of the ants. Independent of its use in ant colonies, dentigerumycin slows the growth of a drug-resistant strain of Candida albicans, a human pathogen that is becoming increasingly resistant to a variety of antifungal drugs.

The chemically prolific actinobacteria involved in these multipartite symbioses are usually Pseudonocardia spp., which attine ants grow within specialized structures on their cuticles. “It may be a coevolutionary arms race between parasites like the virulent and highly evolved Escovopis fungus on the one hand and the remarkably successful ant-fungus-actinomycete mutualism on the other,” says Cameron R. Currie, the microbial ecologist who discovered the ant-actinobacterial symbiosis more than a decade ago and is a member of the dentigerumycin discovery team.

The mutualism between attine ants and actinobacteria is perhaps the most thoroughly studied of all ant-bacterial relationships. Even so, the discovery of dentigerumycin marks the first characterization of a biochemical that strongly suppresses the growth of ant-garden predatory fungi but not the basidiomycete fungus the ants cultivate for food.

Dentigerumycin is not the only chemical game in attine-ant town. “Pseudonocardia evolved a variety of small molecules over their long evolutionary history with fungal-farming ants, with different bacterial strains producing different antibiotics,” Clardy says. “In fact, attine ants are walking pharmaceutical factories,” adds Currie. “Each ant colony maintains a specific bacterial strain and can differentiate between it and foreign actinomycetes.” Finding out how individual ant species and their distinct bacterial sidekicks cope with the specialized parasites that prey on their fungal crops is expected to yield an array of novel antimicrobial agents.

A bonus is that ant colonies also seem to be “miniature biofuel reactors,” Cur-
Plenty of Early Jockeying To Prepare H1N1 Flu Vaccines

In April and May as the H1N1 influenza outbreak spread from Mexico and the United States to much of the rest of the world, public health officials and vaccine manufacturers were actively planning for vaccines to curb that spread. Here are some highlights:

- **GlaxoSmithKline** in mid-May announced plans to make and stockpile a vaccine with adjuvant to protect against H1N1 influenza, pending availability of a seed virus from the World Health Organization (WHO); the company already has orders for 128 million doses from the governments of Britain, France, Belgium, and Finland.
- **MedImmune**, a subsidiary of AstraZeneca, began negotiating with officials at the Centers for Disease Control and Prevention (CDC) in Atlanta, Ga., late in April over developing a vaccine to protect against H1N1 flu.
- **Food and Drug Administration (FDA)** officials in May approved a new U.S.-based facility for producing Fluzone, the Sanofi Pasteur version of the influenza vaccine.
- **Samuel Bogoch**, chair of Replikins, Ltd., in London, U.K., in May said that its synthetic peptide H1N1 vaccine would be made available for testing and evaluation.

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**Uropathogen Seems To Induce Epigenetic Changes Favoring Persistence**

Uropathogenic *Escherichia coli* (UPEC) boost expression of a critical DNA-methylating enzyme in human epithelial cells from the urinary tract, conferring epigenetic changes that make those host cells more vulnerable to chronic and recurrent urinary tract infections (UTIs), according to Darius Bagli of the Hospital for Sick Children in Toronto, Canada, and his collaborators. They reported those findings during the annual meeting of the American Urological Association, held in Chicago last April.

UPEC bacteria are notorious for causing persistent UTIs, with uroepithelial cells being the first targets within the urinary tract of these pathogens. Moreover, the host uroepithelial cells serve as an “internal quiescent reservoir,” Bagli says. Noting that the extracellular matrix can be a potent mitogen to bladder muscle cells, he adds, “We became interested in whether the uroepithelial cell, obviously the first cell to come in contact with bacteria in the subsequent urinary tract infection cascade, may also undergo epigenetic changes in response to infection.”

With a PCR-based assay, Bagli and his collaborators detected a sixfold increase in messenger RNA (mRNA) for DNA-N-methyltransferase-1 (DNMT-1) in vitro in human uroepithelial cells following infection with UPEC, compared to levels of that mRNA in uninfected cells. In turn, DNMT-1 activity is elevated more than 150-fold in such cells, according to the researchers. That enzyme catalyzes DNA methylation and is central to regulating gene expression in such cells. Thus, for example, the increase in DNA methylase activity leads to other changes in these cells, including a decrease in p16 — an important cell-cycle regulator protein.

Those changes appear to disrupt not only p16 but also other cell cycle and DNA repair mechanisms within uroepithelial cells, leaving them more susceptible to persistent UPEC infections, according to Bagli. “We commonly deal with congenital abnormalities of the urinary tract in children...
that are at great risk for harboring and accelerating urinary tract infection,” he says. “If there were some molecular test or state that could be determined from uroepithelial cells (commonly shed in simple urine samples) that would accurately help to predict and differentiate children at high or low risk for infection, then the often-difficult decision as to who might best benefit from surgical correction would become much clearer.”

Sorting out who is more at risk of UTIs also could help to tailor antibiotic treatment or prophylaxis strategies, according to Bagli. A better ability to recognize at-risk groups would be particularly valuable when dealing with patients receiving organ transplants as well as others who are immunocompromised, including those receiving chemotherapy, premature infants, and those in intensive care units or other settings where the risk for infection is high.

“The increase in expression of a DNA methyltransferase is certainly intriguing and raises the possibility that methylation of host DNA promoter regions may occur in association with bacterial invasion,” says pediatrician David Hunstad of Washington University in St. Louis, Mo., who specializes in infectious diseases. “If subsequent studies confirm an increase in DNA methylation, it will open a new area of investigation into epigenetic changes that permit UPEC to persist in the bladder epithelium, and perhaps shed light on the mechanisms by which UPEC might re-emerge to cause recurrences.”

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**SARS Virus Spike Protein Second Cleavage Site Crucial for Species Shifts**

The coronavirus that causes severe acute respiratory syndrome (SARS) has a second cleavage site in the viral spike protein needed for virulence, with that newly identified second site likely allowing the virus to bypass specific receptors and infect cells of different species, according to virologist Gary Whittaker of Cornell University College of Veterinary Medicine in Ithaca, New York, and his collaborators. Their findings could help to explain how this coronavirus, which apparently originated in bats, jumped to civets in Chinese markets and then to humans (see *Microbe*, June 2009, p. 261).

Whittaker’s interest in the SARS spike protein cleavage sites stems from past efforts involving influenza viruses. These viruses contain hemagglutinin sites that are subject to host cleavage during infections. Those sites undergo changes as the viruses shift from being poorly pathogenic to highly pathogenic. This critical flu-based phenomenon inspired Whittaker and his collaborators to search for comparable cleavage site changes in SARS and closely related coronaviruses.

For example, with guidance from the ProP 1.0 server, part of a database that predicts sites along proteins that are susceptible to proteolytic enzymes, they located a second cleavage site in infectious bronchitis virus (IBV), a laboratory-adapted strain of avian coronavirus and a subject for research since the 1940s. “The next logical connection was that IBV’s unusual property of a second cleavage site may be present in other coronaviruses,” Whittaker says. When they analyzed the structure of the SARS virus spike protein, they found a similar proteolytic cleavage site in the same position as in IBV.

The SARS spike protein within the coronavirus envelope regulates receptor binding through its S1 domain and fusion with the host membrane through its S2 domain, according to Whittaker and others. Proteolytic cleavage primes the spike protein in the SARS virus at the S1-S2 boundary, a phenomenon that was recognized several years ago. The second cleavage site in the S2 domain, which the Cornell scientists call S2’, apparently acts in concert with the other site, enabling infectivity and fusion of the virus with the host cell membrane. Cleaving the viral protein at the S1-S2 site promotes cleavage at the S2’ position, triggering membrane fusion. Details appear in the April 7, 2009 *Proceed-
Changes along that second cleavage site somehow allow the virus to jump species, in some cases from animals to humans, according to Whittaker. “It’s still a hypothesis,” he says. “The finding of a second cleavage site opens up lots of theories and lays a foundation to rethink how coronaviruses work in general.” He and his collaborators are searching for similar sites in other coronaviruses.

SARS emerged in 2002–2003, causing severe respiratory illnesses in more than 8,000 individuals and killing 10% of them. Another coronavirus that is equivalent in virulence to SARS eventually will emerge, Whittaker predicts. “It will probably be a different coronavirus from a different animal next time.”

The findings reported by Whittaker and his collaborators are “a carefully executed and beautiful study of the multi-step proteolysis needed for coronavirus entry,” says virologist Tom Gallagher at Loyola University Medical Center in Maywood, Ill. Moreover, their results confirm that entry by SARS following spike protein cleavage is more complicated than earlier assumed. Echoing Whittaker, Gallagher predicts that other zoonotic coronaviruses of equal severity to SARS can be expected to adapt to and infect humans. “If you know the proteases necessary for infection,” he says, “there’s an opportunity to inactivate them with drugs and limit infection.”

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Blood-Like Seepage at Glacier Brims with Metabolic Oddities

A subglacial basin of ancient seawater that is beneath 400 meters of ice making up Taylor Glacier in the McMurdo Dry Valleys in Antarctica is the source of a microbial consortium whose metabolism depends on an extraordinary catalytic sulfur cycle, according to geomicrobiologist Jill A. Mikucki from Dartmouth College in Hanover, N.H., and her collaborators at several U.S. universities and the University of Cambridge in the United Kingdom. Details of their findings appear in the April 17 Science (324:397–400).

Taylor Glacier is the source of one of the most unusual natural anomalies in Antarctica—namely, Blood Falls, a sluggish outflow from the glacier that is ferruginous, meaning it contains iron that turns reddish-brown, the color of blood, when exposed to atmospheric oxygen. The source of this seeping subglacial seawater likely persisted for the past 1.5 million years or more. Radar images of what lies beneath the glacier surface reveal an 80-meter subglacial basin of unknown depth. Microorganisms within that basin are isolated from organic input or oxygen. Thus, they grow in the dark and in brine, according to Mikucki and her collaborators. Moreover, the metabolism of the microbial consortium, based on analysis of samples from the outflow at Blood Falls, depends on a catalytic sulfur cycle, she says.

“Despite the fact that many of the microorganisms are related to known marine microorganisms phylogenetically, their metabolic strategy is quite different,” Mikucki says. “We know that sulfur below the glacier is cycled by biology as evidenced by the functional gene analysis and the sulfur and oxygen isotopic measurements we did, yet sulfate is not reduced to sulfide. Instead, the sulfate is regenerated, or cycled, catalytically. This is quite unusual and has not been observed in natural systems before.”

“The importance of the microbial ecosystem described in the April [17] issue of Science is in the details of the iron and sulfur biochemistry and the possible analogy to the Canfield
Scent Sensors Are New Target for Repellents To Curb Insect Vector-Borne Diseases

Ionotropic receptors (IRs) in antennae of insects are previously unrecognized components of the olfactory system of insects, including fruit flies and mosquitoes, and thus a potential target for repellents that could help in controlling insect-borne diseases, according to Howard Hughes Medical Institute investigator Leslie Vosshall of Rockefeller University in New York, N.Y. Uncovering the link between IRs and insect behavior could prove crucial for manipulating smell and thereby interfering with how insects transmit infectious diseases, she said during a meeting of the Congressional Biomedical Research Caucus in mid-May. Mosquitoes rely on the sense of smell to find humans by catching in on human sweat and carbon dioxide in breath, and this behavior is key to transmission of malaria and other infectious diseases to humans through blood feeding.
media and Congress. Contact with those letters led to 22 cases of anthrax, including five deaths, along with cleanup measures that, for example, cost the U.S. Postal service $1.2 billion to decontaminate several of its facilities, according to Jason Bannan of the FBI Chemical-Biological Sciences Unit in Quantico, Va., and a participant in the ASM plenary session. “FDA never had a case like this before,” he says.

No spore-containing letter was recovered from the first attack that led to the death of a photojournalist in Boca Raton, Fla. However, investigators recovered spores as part of a granular, white powdery material from an envelope involved in the second incident. Bannan describes it as a “crude prep,” in part because it also contained Bacillus subtilis. Additional material from other letters to then-Senator Tom Daschle (D-SD) and to Senator Patrick Leahy (D-VT) in October 2001 appeared “more refined,” was “beige” instead of white, and contained no spores other than B. anthracis.

The FBI quickly requested outside microbiologists to help in analyzing those materials. The available “research assays . . . didn’t meet forensic standards,” says Keim who, with his collaborators at NAU, worked closely with the FBI, as did other outside groups of microbiologists and investigators with other expertise. Moreover, efforts to develop such assays were complicated by the strictly clonal biology that B. anthracis follows during replication.

Those facts soon led microbial and molecular forensics investigators into conducting genomics-level analyses, according to Jacques Ravel, now at the University of Maryland School of Medicine and Institute for Genome Sciences in Baltimore, Md. A more conventional phenotypic analysis supplemented those genomic-level efforts, leading another group of microbiologists at USAMRIID, who were working with the FBI and others on the anthrax investigation, to take advantage of distinct B. anthracis “morphotypes” that could be observed on growth plates. Those morphotypes vary not only by colony appearance but also in sporulation efficiencies and in telltale mutations at a rare “hot spot” within the otherwise stable genome of this species.

That information became the basis for a PCR screening assay for B. anthracis specimens that then was validated at Commonwealth Biotechnologies (CB) in Richmond, Va., and the Midwest Research Institute in Palm Bay, Fla., to ensure that such testing could meet forensics standards applied by U.S. courts. By 2007, the “highly specific” PCR assay identified several samples during a “blinded” analysis that included “seized materials,” Bannan says. Ultimately, the PCR-based analysis along with other information from the criminal side of the investigation indicated that the anthrax-causing specimens from the 2001 letters derived from stocks produced in at least two separate batches, contaminated with B. subtilis at least once, but when and how remain unknown. “We don’t know the process used,” Bannan says. “We never found the equivalent B. subtilis at USAMRIID in any of the evidence that we had.” Efforts to trace the source of that bacterial contaminant “didn’t lead anywhere,” adds Keim.

Early reports suggested that the

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**Sludge Bacteria Prove Apt at Degrading Cholesterol**

Gordonia cholestrolivorans, a bacterial species isolated from sewage sludge, can actively degrade cholesterol in such settings but might be used to make or modify novel types of cholesterol derivatives for medical applications, according to Oliver Drzyzga and his collaborators at the Universidad Complutense de Madrid, Spain. Because some Gordonia species are human pathogens, it is unlikely that these bacteria will ever be used directly to control cholesterol levels in humans, Drzyzga points out. “We are trying to work out exactly how G. cholestrolivorans metabolizes cholesterol so that we can identify and construct metabolically engineered strains that are more rapid and effective in breaking down cholesterol.” Details appear in the May International Journal of Systematic and Evolutionary Microbiology.
spores were “weaponized,” possibly with “silica.” However, later analysis determined that the spores were not coated with silica, although silicon was found within—not outside—the coat of spores used in the attacks, according to Joseph Michael of Sandia National Laboratory in Albuquerque, N.M. About two-thirds of the spores contain that silicon “signature,” he says. Attempts to grow fresh spores with silicon to determine whether it also would locate within the spore coat led to “variable” results, Bannan adds. “We don’t understand why there is a varying degree of silicon from one batch to another.”

Other questions regarding physical properties of the spores similarly remain unexplained. Asked whether the spores were milled, Bannan points out that B. anthracis spores in letters went through rollers in automated postal sorting equipment that subjected them to high pressures. “It’s a high-energy process, and [spore] plumes went up 30 feet [about 10 m] from the mail sorters,” he says. How those spores looked beforehand or whether they were pulverized after being dried and before being inserted into envelopes is not known.

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Novel Means for Spreading Resistance Traits among Gram-Negative Pathogens

A newly recognized mobile element named “integron mobilization unit” (IMU) is a novel means for spreading antibiotic resistance genes, thus adding to the roster of molecular mechanisms for disseminating resistance traits to a broad variety of gram-negative bacterial pathogens, according to Johann D. Pitout of the University of Calgary in Calgary, Alberta, Canada, Patrice Nordmann of INSERM in Paris, France, and the nearby Hopital de Bicetre, and their collaborators. Although not a conventional transposon, the IMU behaves much like a miniature inverted transposable element (MITE), marking the first time that such an element was found to mobilize clinically relevant resistance genes, the researchers point out. This instance also appears to be the first report of the GES-5 class A carbapenemase being detected in North America. Details appear in the June 2009 Antimicrobial Agents and Chemotherapy (53:2492–2498).

This story began to take shape in March 2006, in Calgary, where Pitout and other clinicians isolated Enterobacter cloacae, a gram-negative pathogen, from a patient with severe pneumonia who was being treated at an acute care center. The isolate contains a plasmid carrying genes conferring resistance to many antibiotics, including all β-lactams, tetracycline, fluoroquinolones, and sulfonamides. However, the pathogen remains susceptible to gentamicin, amikacin, and colistin. Using PCR primers that are specific for β-lactamase genes, the researchers “identified the gene responsible for the powerful β-lactamase GES-5,” Nordmann says.

In other settings, that gene tends to associate with structures called integrons, which are genetic elements that capture, carry, and redistribute genes. Recognizing those properties, Nordmann says, convinced him and his collaborators that they should “identify the latter structure in detail.” Although other integrons disseminate genes only when situated within transposons, this integron proved to be free of any transposon, he says. Moreover, the gene cassette containing the β-lactamase GES-5 gene has other peculiarities. For example, the cassette also contains truncated versions of an integrase gene as well as another gene that ordinarily are intact within class 1 integrons.

The IMU itself consists of a pair of identical, 288-bp sequences at opposite ends of this genetic complex. This overall oligonucleotide segment does not encode a peptide and is not congruent with any other known oligonucleotide sequence. However, an initial partial segment that contains 38 bp is 95% identical with the end of an insertion sequence on a transposon within a plasmid of Shewanella oneidensis.

This IMU can readily transpose resistance genes from one species into another—for example, from Escherichia coli to Pseudomonas aeruginosa, according to Nordmann and Pitout. Although the IMUs resemble MITEs, which are found in bacterial, archaeabacterial, and eukaryotic genomes, MITEs typically regulate messenger RNA molecules and were not believed to mobilize antibiotic resistance genes, the researchers point out.

The IMU is worrisome because it provides yet another means for disseminating drug resistance among potent gram-negative bacterial pathogens, according to Rafael Canton of Hospital Universitario Ramon y Cajal in Madrid, Spain, who was not involved in the research. Its “association with a broad-spectrum plasmid enhances its significance, as it can be transferred among different organisms,” he says. “Future epidemiological studies will demonstrate the potential spread of this newly identified platform...and could shed further light on the spread of resistance.”

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