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-hydroxyxypiperazic 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-
Favoring Persistence

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...

Marcia Stone
Marcia Stone is a science writer based in New York City. More of her work can be seen on www.mstoneworks.net

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.
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Vaccines

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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 Whitaker, 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.”

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

Blood-Falls, a ferruginous outflow from under Taylor glacier in Antarctica, arises from an unusual microbial ecosystem which may provide clues about terrestrial conditions of 500 million to 1 billion years ago. (Photo courtesy of Jill A. Mikucki, Dartmouth College, Hanover, N.H.)
Thus, the conditions during the Snowball Earth episode could be much like those now found at Blood Falls in the Antarctic. In both cases, thick ice lowers organic matter that microorganisms produce.

The Mikucki “observation that Fe can persist in a system even with high sulfate concentrations . . . would imply that something other than sulfate concentration (or, in my way of thinking, sulfate flux to the oceans, which isn’t exactly the same) changes could promote a flip between iron-rich and sulfidic ocean conditions,” Canfield says. “[She and her collaborators] argue that organic matter availability may be just the trigger. I think this is an interesting idea and may have relevance in some systems like the one explored in their paper. However, . . . the flux of organic carbon to the deep ocean far exceeds the flux of iron, so, for the Mikucki model to work, the primary productivity of the Precambrian oceans must have been orders of magnitude less than it is now. There’s nothing in the geologic record to suggest that this was true.”

“Energetics would favor iron reduction as organic matter became limited,” Mikucki says. “Our results demonstrate that the absence of sulfate is not required for a system to accumulate Fe(II). The brine below the Taylor Glacier is ferruginous despite significant concentrations of sulfate, thus organic matter availability is an important factor in the ‘choice’ of respiratory pathway.”

Barry E. DiGregorio
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Questions Linger over Science behind Anthrax Letters

Despite the outcome in mid-2008 of the Federal Bureau of Investigation (FBI) probe into the deadly and disruptive anthrax attacks of 2001, the FBI in May arranged for the National Academy of Sciences (NAS) to review the microbial and other forensic efforts that bureau officials coordinated as part of its broader investigation. It led FBI officials to conclude that microbiologist Bruce Ivins of the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) at Fort Detrick in Frederick, Md., was the sole culprit behind the letter-based attacks (Microbe, October 2008, p. 453).

Nonetheless, skepticism persists, as is evident not only from the forthcoming NAS review but also during the plenary session, “The Science behind the ‘Anthrax Letter’ Attack Investigation,” convened as part of the 7th ASM Biodefense & Emerging Diseases Research Meeting, held in Baltimore, Md., last February, and during the news conference that followed. “Everybody is frustrated by the lack of closure,” says plenary session participant Paul Keim of Northern Arizona University (NAU) in Flagstaff.

Soon after the World Trade Center in New York, N.Y., and the Pentagon outside Washington, D.C., were attacked on September 11, 2001, letters containing spores of Bacillus anthracis were sent to members of the news

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 cueing 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.

ocean, a sulfidic ocean, that may have existed on Earth [about 1] billion years ago,” says Christopher McKay, an astrobiologist from the National Aeronautics and Space Administration Ames Research Center in Moffett Field, Calif., who also studies microbial diversity in Antarctica. McKay refers to Donald Canfield of the University of Southern Denmark in Odense and his collaborators, who about a decade ago challenged mainstream views about when oxygen appeared in abundance on Earth. They argue that the oceans remained anoxic and were also sulfidic until as recently as 0.54 billion years ago, when according to this view the planet was frozen, a stage known as Snowball Earth.

“The results of Mikucki [and her collaborators] remind us that we should never forget the role of organic matter in the story,” says biochemist Timothy Lyons at the University of California, Riverside. “I am particularly intrigued by their suggestion that bacterial hydrogen sulfate reduction can stop short of hydrogen sulfide and were also sulfidic until as recently as 0.54 billion years ago, when according to this view the planet was frozen, a stage known as Snowball Earth.

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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 several years earlier at USAMRIID for an aerosol challenge in anthrax vaccine studies, he says.

Based on that and other information from more conventional lines of evidence, FBI investigators concluded that Ivins, who died following a drug overdose in July 2008, produced spores from those stocks for the 2001 anthrax attacks.

Despite that painstaking analysis and the unequivocal conclusions put forth by FBI officials, doubts linger over some matters that are mainly scientific as well as others that intersect with the broader thrust of the investigation. For instance, none of the microbiologists, including Bannan and similar specialists at FBI, was privy to other evidence, including lab records from USAMRIID, that their FBI colleagues collected. “I know nothing of that information,” he says. “I’m a microbiologist, and was not involved in the seizure of evidence.”

Other lingering questions focus on more purely scientific issues, some of them pertaining to how the lethal bacteria were handled. For example, USAMRIID held B. anthracis in aqueous suspensions, not as spores. Presumably, the spores sent via letters were 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.

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**Gordonia cholesterolivorans**
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.

**Jeffrey L. Fox**

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

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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.”

**David Holzman**

David Holzman is the *Microbe* Journal Highlights Editor.