Current Topics

Pigments of Pathogens Might Provide Golden Antimicrobial Opportunities

The carotenoids that make carrots orange also produce the golden hue of *Staphylococcus aureus*. Such pigments appear to be a golden armor, shielding this pathogen from oxidants that mammalian neutrophils release, thus accounting for some of this microorganism's virulence, according to Victor Nizet at the University of California, San Diego (USCD) and his collaborators. Perhaps these colorful virulence factors will provide a new golden opportunity for battling staph infections, which are becoming resistant to standard antibiotics.

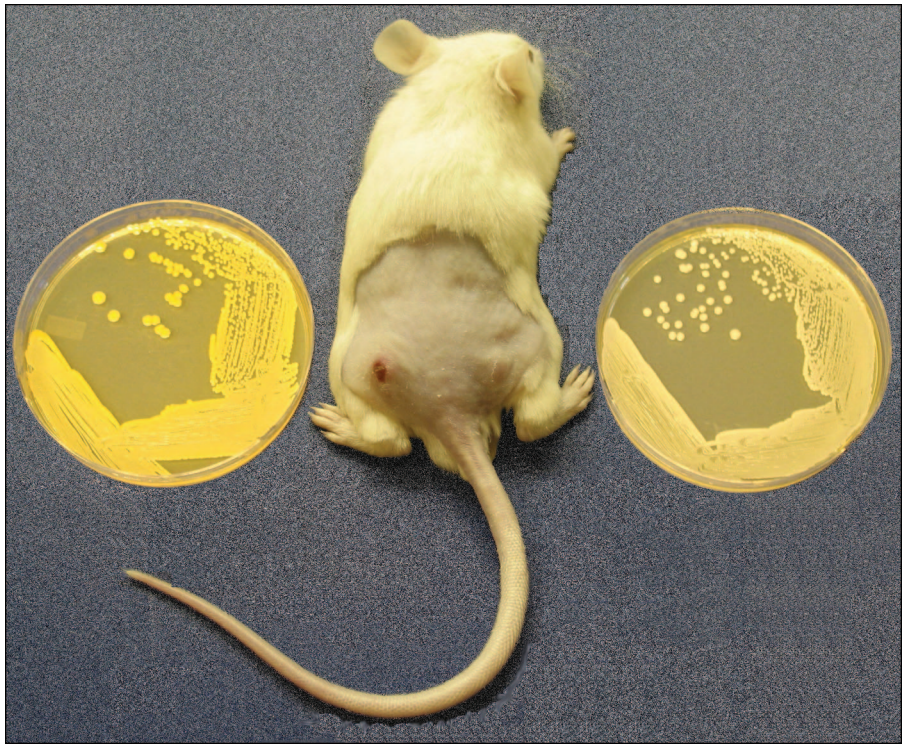
The UCSD researchers knocked out a key gene for carotenoid biosynthesis, generating colorless *Staphylococcus* mutants, and also inserted carotenoid genes into normally colorless *Streptococcus pyogenes*, making it turn yellow. "We moved the first two genes in the pathway from staph into *pyogenes*. That was enough to create some yellow pigments, although not the rich-colored pigments that staph has," Nizet says.

Making staph colorless renders it more susceptible to killing by human neutrophils or by hydrogen peroxide in vitro. When injected into the skin of mice, the colorless staph mutant does not produce characteristic abscesses, whereas large lesions grow within four days after injections of wild-type *S. aureus*. Meanwhile, the yellow strep transformant becomes more resistant to oxidants and neutrophil killing. It also gains the capacity to produce abscesses when injected into mouse skin, according to the UCSD researchers, whose study appears in the July 17 issue of *The Journal of Experimental Medicine*.

Another carotenoid pigment gives Group B *Streptococcus* (GBS) a distinctive orange tinge, according to George Liu, a Nizet collaborator. While culturing GBS mutants lacking carotenoids, Liu noticed that these bacteria fail to grow in test tubes that are slightly contaminated with bleach. Liu also recognized that white blood cells release hypochlorite, the active ingredient of bleach, as a means for killing bacteria. Wild-type GBS survives inside macrophages and neutrophils. However, macrophages and neutrophils, as well as hydrogen peroxide, hypochlorite, and singlet oxygen, kill carotenoid-deficient GBS mutants, according to a report by Liu, Nizet, and their colleagues in the October 2004 *Proceedings of the National Academy of Sciences*.

"Anyone working with a colorful

FIGURE 1



Pigmented staph mutants (left) cause abscesses in mice, whereas mutants without pigment are less virulent and more susceptible to immune defenses (image courtesy of Victor Nizet, University of California, San Diego).

pathogen might ask whether the pigment serves a defensive function that contributes to disease," Nizet says. Other pigmented candidates include bacteria such as *Burkholderia cepacia* and *Serratia marcescens* and the fungus *Aspergillus fumigatus* that make carotenoids or melanin with antioxidant properties. A strategy of blocking pigment production could lead to development of novel antimicrobials that disarm rather than kill pathogens and are targeted to special features of carotenoid pathways, which vary widely among bacteria.

When stripped of their pigmented armor, pathogens may succumb more readily to natural immune defenses, perhaps without developing resistance, speculates Nizet. "When you give a bacterium a life-or-death challenge, there's huge selection pressure to evolve resistance," he says. For almost every antibiotic ever created, "some bacterium has evolved resistance to it."

"This pigment has always been a curiosity, now we have a function for it," says Paul Sullam, an infectious disease specialist at the University of California, San Francisco. Calling this finding "an intriguing new target" for drugs, he points out that key challenges include making agents that are specific for carotenoid pathways in the organism, yet are safe in humans. When it comes to drug design, he adds, "many are called, but few are chosen."

Carol Potera
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FDA Halts Use of Specific Antibiotic in Agriculture

In an effort to curb development of antibiotic resistance, Lester Crawford, who is Commissioner of the Food and Drug Administration (FDA), announced late in July a halt to further use of the antimicrobial drug enrofloxacin for the purpose of treating bacterial infections in poultry. That drug, distributed as Baytril by Bayer Corp., belongs to the larger class of fluoroquinolones. Such antibiotics are used widely for treating illnesses in humans, including foodborne *Campylobacter* infections in humans, whose resistance to such treatments has "increased significantly," according to FDA. The ban, originally proposed in October 2000, marks the first time the agency has withdrawn an antibiotic from agricultural use because of concerns about its impact on resistance among drugs used for treating humans. Bayer, which had argued that Baytril is critical for poultry production and petitioned the agency not to ban its use, in September agreed to withdraw the product. Meanwhile, many top poultry producers have announced that they no longer use these drugs in chickens produced for human consumption, according to the Keep Antibiotics Working Coalition, which commends FDA for the enrofloxacin action.

National Academies: Bolstering Public Health Key to Biosecurity for Russia

With biosecurity forming the backdrop, a committee of the National Academies (NA) late in July named public health surveillance, research on pathogens and in biotechnology, and programs to cultivate human resources the crucial "pillars" for supporting U.S.-Russian collaborative efforts while revitalizing Russia's wavering public health infrastructure.

The NA report, "Biological Science and Biotechnology in Russia: Controlling Diseases and Enhancing Security," calls for creating a bilateral "commission on infectious diseases," with experts from the United States and Russia. Its purpose would be not only to identify "high-payoff research areas" but also to re-energize Russia's capacities to cope with infectious diseases. "Russia has made strides. . .but financial constraints have impeded its ability to join with the United States and the international community in major initiatives to control diseases and to enhance biosecurity," says NA committee chair David Franz of the Midwest Research Institute in Kansas City, Mo.

Enhancing biotechnology in Russia while improving its public health surveillance and response system, research on pathogens, and human resources for conducting these activities will provide "increased mutual confidence" involving research in "sensitive areas," according to the report. It cites Russian estimates of a domestic market for human drugs amounting to $5 billion in 2005 and perhaps $50 million in sales per year for human vaccines. It also says that there are little or no data available to track sales of such products for plants and animals.

Russian scientists, who once worked in bioweapons programs and developed a "world-class expertise," could become more fully engaged in meeting pressing public health needs if these recommendations were implemented, says NA committee member Christopher Howson of the March of Dimes Birth Defects Foundation in White Plains, N.Y., adding: "Our hope is that Russians might be encouraged in developing the biotechnology sector not only for their own population but for exports."

However, the report points out, such goals remain "a long way off" until the country develops consistent tax and reward-oriented intellectual property policies and also streamlines its capacity for evaluating biotech products and facilities. Further, for re-

search on pathogens to progress more effectively, the Russian government and foreign partners will need to provide better financial support through a "fair and open competitive process." Facility and equipment upgrades also are needed. "Most labs look like the 1950s," Franz says, noting that, despite such pressing needs, it is "difficult for us to support infrastructure." In any case, equipment needs generally play a subordinate role to strengthening programs and attracting and retaining personnel with scientific expertise.

The NA report also seeks eventually to revamp Russia's extensive network of surveillance centers that, for many years, also concealed an active bioweapons development program. In a related development, Raymond Zilinskas of the Monterey Institute of International Studies in Monterey, Calif., recently completed a study of components of this same system, with its focus not on Russia but other countries of the former Soviet Union. He and several collaborators described those efforts during a symposium, "The Anti-Plague System of the Former Soviet Union: Biological Weapons Proliferation Potential and Promises for International Public Health," at the 105th General ASM meeting last June in Atlanta, Ga.

The sprawling "anti-plague" system dates to the 1920s and contains a hodgepodge of public health, agricultural, railroad, military, and other components, according to Zilinskas. "What we worry about are proliferative threats," he says. Professional staffs are "grossly underpaid" and personnel, including at sites near Iran, could be hired away, many culture collections are "unsecured," and some lab equipment could too readily be "diverted" to bioterrorist uses. Besides surveying the system and uncovering details of its history, Zilinskas and others are—much like recommendations in the NA report on Russia—trying to "bring up the public health capability" of this parallel system throughout many of the former Soviet states.

Jeffrey L. Fox
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Genomes of Bacteria Living in Ants Deteriorated Long Ago, then Stabilized

Blochmannia bacteria have been living inside *Camponotus* and related ant genera for 30 million years or more, but the current genomic stability of this symbiotic microbe belies ancient turbulence, according to Jennifer Wernegreen from the Marine Biological Laboratory (MBL) in Woods Hole, Mass., and her collaborators, who reported their findings in *Genome Research* in August (http://www.genome.org/cgi/doi/10.1101/gr.3771305). The overall picture indicates the bacterial genome deteriorated early, losing many gene functions, but then stabilized, she says. "This genomic stability may prevent the reacquisition of those lost functions or the evolution of new ones. In addition, rapid protein evolution seems to degrade the genes that remain." The genome of *Blochmannia pennsylvanicus*, which associates with the black carpenter ant *Camponotus pennsylvanicus*, contains only 791,654 nucleotides. Remarkably, although this endosymbiont diverged from the carpenter ant mutualist, *B. floridanus* between 16 and 20 million years ago, all 635 genes shared between these two bacteria are completely conserved in terms of order and strand orientation, the MBL researchers report. Such striking genomic stability is also seen in *Buchnera*, an aphid mutualist, thus suggesting that genome "stasis" is a "general feature of insect mutualists." The MBL researchers also showed that *Blochmannia* amino acid sequences diverged approximately 50 times more quickly than do those in proteins in free-living bacteria, suggesting that endosymbionts may be "more tolerant of amino acid changes when they first become associated with their hosts."

Pair-Wise Genomics Provides Novel Approach for Microbial Taxonomy

When doing taxonomy, modern microbiologists ordinarily depend on 16S ribosomal RNA (16S rRNA) sequences to determine genetic relatedness. Yet this reliance on one gene among thousands may very well have shortcomings when it comes to comparing and ranking organisms. To address this challenge, Konstantinos T. Konstantinidis and James M. Tiedje of Michigan State University (MSU), East Lansing, propose a new system for measuring "evolutionary distances" between pairs of microorganisms. Their new approach to microbial taxonomy entails using the average amino acid identity (AAI) among all genes that are conserved between two organisms for which genomic sequence information is available.

This approach provides "more accurate and more robust" comparisons of microorganisms than does the 16S rRNA-based system, Konstantinidis and Tiedje report in the September 2005 issue of the *Journal of Bacteriology* (**187:**6258–6264.). The AAI system is capable of measuring relatedness of closely related genomes, even from different strains of the same species, something that the 16S rRNA approach cannot manage, they say.

The MSU researchers evaluated and refined their scheme by comparing genome pairs among 175 microbial species and strains. In doing so, the researchers show that the system holds up despite the fact that the numbers of conserved genes vary between different pairs of genomes, and despite the fact that conserved genes from distantly related organisms fall into different classes from some of those that are conserved between closely related species. For instance, between closely related genomes, "auxiliary" genes such as those involved in metabolism or mobility are often conserved along with "housekeeping" genes such as ribosomal genes and DNA/RNA polymerases, whereas only the latter are conserved among genomes of distantly related organisms.

Some of the power of AAI lies in a certain consistency of results that is maintained regardless of genetic distance. The researchers calculated that in all pair-wise comparisons—175 × 175, or 30,625 comparisons in all—the congruence of the sequences of the "great majority (more than 70%)" of the homologous genes fell within 8% of the genome average. In other words, hypothetically, if the average sequence congruence between two genomes were 50%, more than 70% of the gene pairs would have sequence congruences that would fall between 42% and 58%. That finding remains "consistent regardless of the absolute genetic distance between the genomes compared," the authors write.

In exploring and testing the AAI method, the MSU researchers find that hierarchies within the current taxonomic system can prove somewhat blurry because of frequent overlaps between organisms with adjacent rank. AAI can be used, however, to sharpen the evolutionary boundaries between ranks and correct some of the obvious irregularities in the current taxonomic system, rendering it more uniform and predictive.

Further, the researchers find so much diversity among bacteria that several phyla and a few classes of bacteria appear to be as distantly related as the bacteria are from *Archaea*.

One operational problem for the new method is that it can be used only for ranking organisms whose genomes are sequenced. But multilocus-sequencing typing (MLST) could provide one way to circumvent that data-intensive requirement. For instance, a MLST-based phylogenetic reconstruction proved "very congruent with the AAI-based" ranking when the MSU researchers compared seven high-draft *Burkholderia* genomes and seven genes for this purpose.

Beyond these fundamental analyses and applications, the AAI method may well find practical use in clinical settings. Konstantinidis says that its ability to distinguish closely related microbial strains could make it valuable when comparing potentially innocuous with virulent strains of pathogenic microorganisms.

These findings are "very exciting," and raise several important issues, says microbial ecologist James Staley of the University of Washington in Seattle, who was not involved in the development of this new means for classifying microorganisms. Beyond its implications in basic microbiology, he adds, this approach could prove useful in practical settings, even for analysts faced with bioterrorism threats. "If you have isolated an organism, you would like to know what it is immediately," he points out.

David Holzman
David Holzman writes from Lexington, Mass.

Adding Molecular Probes to AFM Probes To Reveal Microbial Adhesin Behavior

By adding customized molecular "tips" to probes, researchers can now use atomic force microscopy (AFM) to analyze how specific adhesin molecules are distributed and function on the surfaces of bacteria, including pathogens such as *Mycobacterium tuberculosis*, according to Yves Dufrene of Universite Catholique de Louvain in Louvain Pasteur de Lille la Neuve, Belgium, and other collaborators in Europe and the United States. Cells of this pathogen produce a heparin-binding hemagglutinin adhesin that they analyzed after attaching heparin molecules to the probe, where they serve as stand-ins for the heparan sulfate proteoglycans that this bacterial protein ordinarily binds to on host epithelial cells. Through such AFM-based measurements, they determined that, instead of being randomly distributed along the bacterial surface, the adhesin concentrates in "nanodomains." This approach, besides being applicable for mapping and doing functional analyses of "virtually . . . any microbial adhesin molecule," also could be adapted to studying other subjects such as "biofilm formation," according to their report in the July 2005 issue of *Nature Methods*.

Therapy with Phage: Mirage or Potential Barrage of Products?

"Why does phage therapy get such a bad rap?" asks William Summers, who convened the symposium "Phage Therapy: New Life for an Old Idea?" during the 105th ASM General Meet-

Computer Models Help Frame Flu-Containment Strategies

Preventing an influenza pandemic would require implementing several public health measures, particularly antiviral drugs, soon after the first cases appear during an outbreak, according to two similar efforts to model such an epidemic. Those efforts, one led by Neil M. Ferguson, a computational biologist at Imperial College in London, England, and the other by Ira M. Longini, Jr., a biostatistician at the Emory University Rollins School of Public Health in Atlanta, Ga., both used data from Thailand to develop scenarios involving early outbreaks of an equally virulent but more readily contagious version of the H5N1 influenza virus that is now circulating in several countries in Asia. The former model suggests that an international stockpile of 3 million courses of flu antiviral drugs, combined with other interventions, could contain a pandemic, according to Ferguson and his collaborators, who published their findings in the August 3, 2005, issue of *Nature*. According to the second modeling effort, whose results appear in the August 5, 2005, issue of *Science*, effective containment strategies include giving antiviral medication to people in the same social networks, administering a vaccine before an outbreak, even one that is not so well matched to the strain that emerges, and also quarantining the houses or neighborhoods of infected people.

ing in Atlanta, Ga., last June. "Whether we can get the holy grail of systemic therapy with phage is still very much an open question," says Steven Projan of Wyeth Ayerst Research in Cambridge, Mass., who joined a free-flowing discussion toward the end of the symposium. Although known for his skepticism on this subject, he also contends that "there's no excuse for not funding research on phage therapy."

The field of phage therapy has a tough history, according to Summers. Decades ago when the therapeutic future of phage appeared bright, antibiotics came along and helped to chase them away—a task made easier during the Cold War era when scientists in the West discounted much of the phage research then being done in the Soviet Union. Further, renowned molecular biologist and textbook author Gunther Stent declared that phage therapy "didn't work," and his influence proved substantial. Further, well-behaved coliphage became a major focus of U.S. and European research even though its behavior little reflects "what happens in the wild," where disease occurs and therapy must be made to work, points out Ryland Young of Texas A&M University in College Station.

Despite these and other stumbling blocks, phage therapy—broadly defined—is facing brighter prospects in several settings, including for agricultural and food uses and also as a means for curbing life-threatening diarrheal outbreaks in Bangladesh and elsewhere in Asia. Agricultural applications are attractive because they provide a means for "making regulators comfortable before [comparable projects] go into clinical testing," points out symposium participant Kishore Murthy of GangaGen Life Sciences in Ottawa, Ontario, Canada. Phage-based products intended for agricultural and food applications also tend to cost less and involve "lower burdens," adds Alexander Sulakvelidze of the University of Maryland, Baltimore. "The cost for us is about $10 million for agricultural applications," he says. "So it shouldn't take so much money, but it also won't make as much money as would a new antibiotic."

Another potential stumbling block is how the public will react to phage in, say, food products. "It could be seen as scary, and the public is not aware that there already are a lot of viruses in and on food," Sulakvelidze says. "There will be somebody who objects, but many people are not opposed." Murthy says that responses to a recent survey were "quite positive," and Young notes that phage are considered "green," meaning environmentally friendly, in some circles.

One such seemingly green approach is aimed at reducing *Escherichia coli* O157 levels among cattle. That pathogen is the company's "primary target" for phage in the agricultural sector, Murthy says. The strategy is to treat cattle with phage on feedlots and at processing plants "to reduce the load" of those bacteria within the flora of the animals as well as the risk of "cross-contamination" between animals. Secondary uses could include dairy operations where phage treatments might reduce contamination in manure and, through a "ripple effect," water that becomes contaminated because of runoff from fields on which such manure is spread.

Encapsulated phage particles are mixed with feed for animals that are brought to lots, and these additives raise phage levels in the animals over about a week, according to Murthy. Although dosing is "not optimized," small-scale tests with single doses of phage can reduce shedding from cattle of *E. coli* O157 to below the detection limit, he says. "The phage are highly

specific for O157, there is no change to the rest of the flora, and the cattle show no deleterious effects." Processors are showing "interest" in the product—in part, reflecting their fear of economic losses from being shut down if their plants become contaminated with *E. coli* O157, he adds.

On the clinical front, phage are being developed as a "natural means for fighting diarrheal diseases" caused by *E. coli,* according to Harold Brussow of Nestle Research Center in Lausanne, Switzerland. Working in Dacca, Bangladesh, where *E. coli* is one among several potent agents of diarrheal disease, Brussow and his collaborators locally isolated a T4 phage that is effective in destroying that bacterial genome during replication and also proves effective in vivo, lysing such bacteria in infected mice.

"We can only shift the equilibrium with phage against *E. coli* in the gut . . . but that might be enough in a clinical situation," he says. If clinical safety trials go well, plans call for clinical "empirical efficacy trials" to determine whether these phage can help to control local outbreaks of diarrhea.

Jeffrey L. Fox

Plumbing Commensal Reservoirs of Antibiotic Resistance

"A few years ago you couldn't publish in *Antimicrobial Agents and Chemotherapy* about commensals," says Marilyn Roberts of the University of Washington, Seattle, who spoke during the symposium, "Commensal Reservoirs of Antibiotic Resistance," convened during the 105th ASM General Meeting in Atlanta, Georgia, last June. "Now you can." Roberts and other symposium participants contend that commensal bacteria may be the dominant reservoir for antibiotic resistance.

When Roberts and her collaborators recently investigated fresh and saltwater aquaculture facilities in Chile, the second-largest producer of salmon in the world, they found antibiotic resistance in a whopping 72% of bacterial commensals associated with salmon. These high values appear all the more extraordinary because representatives from the Chilean facilities said that the antibiotic and growth enhancer oxytetracycline had not been used for at least the previous six months.

Bacteria in soils also carry surprisingly high levels of antibiotic resistance genes, according to James Tiedje of Michigan State University (MSU) in East Lansing. Tiedje and his collaborators analyzed DNA in soil samples for the presence and prevalence of tetracycline (*tet*) resistance genes—investigating the impact of agricultural uses of such drugs on bacteria found in soil. For example, one week after farm manure is spread on a test field, the *tet(36)* antibiotic resistance gene is amplified in soil samples but subsequently declines.

However, when tetracycline is added to such samples, the prevalence of this gene increases because susceptible bacteria die off or resistant bacteria increase. Either way, the soil can serve as a reservoir of tetracycline-resistant bacteria, according to Tiedje. One approach to dealing with antibiotic resistance in commensal bacteria would be to consider them as pests and then to develop appropriate pest management programs, he says. Awareness gained by learning more of the nature of the problem and its scope will drive the policy.

Another MSU researcher, Thomas Whittam, described the diversity of antibiotic resistance markers found in commensal *Escherichia coli*. For example, he and his collaborators surveyed antibiotic and other genetic markers in *E. coli* that they isolated from beach sand at a site along Lake Michigan. They found extensive genetic diversity among these isolates along with evidence of extensive genetic exchanges in the face of a scarcity of nutrients. By trading genes to improve their chances of survival, these bacterial also appear to be disseminating antibiotic resistance genes, according to Whittam.

"Bacteria we don't pay attention to are important in antibiotic resistance," says symposium organizer Stuart Levy of Tufts University Medical School in Boston, Mass., and a founder of the Alliance for the Prudent Use of Antibiotics (APUA). "We're stepping back to look at. . . commensals, focusing on a group of genes that are a health threat and giving us a focus that we hope will predict and prevent resistance problems." Through APUA, he and other collaborators are collecting antibiotic resistance data for microbes found in varied global environments. "Why wait for genes to appear as a clinical problem, when the genes are in the natural environment?" he asks. "Why not go look for them?"

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