CHESAPEAKE QUARTERLY

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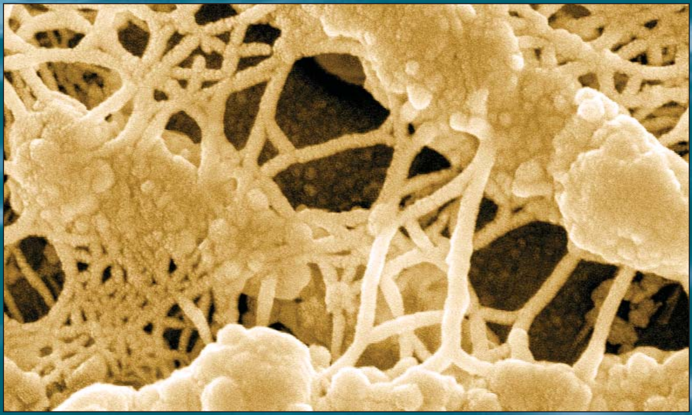
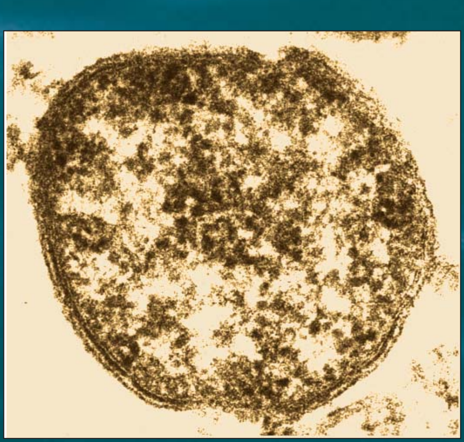


Lessons Aquatic Microbes Can Teach



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CHESAPEAKE QUARTERLY

June 2006

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Cover photo: *Denizens of a world beyond human perception, bacteria in aquatic environments can perform complex ecological feats. (Top right)* Sacchariphagus degradans 2-40, PHOTOGRAPH BY RONALD WEINER; *(middle)* Silicibacter TM1040, PHOTOGRAPH © DENNIS KUNKEL MICROSCOPY, INC; *(bottom)* Dehalococcoides ethenogenes, PHOTOGRAPH BY STEPHEN ZINDER. ***Photo on opposite page:*** *Another image of* Sacchariphagus degradans 2-40, *a very effective degrader of marine plants and chitin, found in animal exoskeletons.* PHOTOGRAPH BY RONALD WEINER.

What the Eye Can't See

Bacteria often get a bad rap as bearers of disease and pestilence. But microbial forces in the environment, orders of magnitude smaller than the human eye can see, can maintain or improve the ecological status quo, sustaining the Earth's delicate balancing act. Microbes live in some of the most unlikely environments around — the hottest, deepest, coldest, and most toxic. Many break down noxious compounds in the course of meeting their own nutritional needs. And the more they are exposed to these compounds, the more efficient they become.

In the process of going about their metabolic business, many microbes inadvertently join in the fight against contaminants of human origin. Others break down tough-to-dispose-of natural products, such as crab shells and giant kelp, keeping the ocean floor debris-free and recycling copious quantities of organic carbon. Through the powers of symbiotic association, still other bacteria protect their charges against disease or infection with antibiotic assets. Some may prove valuable sources of drugs that can fight human diseases.

By understanding what microbes do naturally to preserve Earth's equilibrium, can we harness their power to clean up our own messes? Can we help them do what they already do best? In this issue of *Chesapeake Quarterly*, we dip into the world of microbial ecology, drawing upon the work of several different researchers to explore recurrent themes in microbial interactions, themes such as competition, symbiosis, and adaptation that we more commonly associate with the macroscopic world — the world that we can see.

This issue explores recent discoveries by a diverse group of scientists devoted to uncovering the secret capabilities of the microbial underworld. Jennifer Becker, a bioenvironmental engineer at the University of Maryland College Park (UMCP), studies the ecological interactions among bacteria that break down a chemical contaminant associated with the dry cleaning industry, called PCE. Kevin Sowers, at the University of Maryland's Biotechnology Institute (UMBI) in Baltimore, researches microbes that degrade related contaminants called PCBs, which wend their way through the food chain, often affecting top predators in the marine ecosystem, such as birds and fish. Robert Belas, also from UMBI, studies a group of bacteria with antibiotic properties — a group that may protect coral reefs from the diseases that cause bleaching. UMBI's Russell Hill works to identify drugs from the sea that come from bacterial sources. And Ronald Weiner, also from UMCP, studies microbes with tremendous powers of degradation, capable of recycling all sorts of marine matter.

When James Lovelock wrote *Gaia: A New Look at Life on Earth* in 1979, his theories met with great skepticism. To many, the premise that the "entire range of living matter on Earth, from whales to viruses, and from oaks to algae," may function as a single living entity, capable of "manipulating the Earth's atmosphere to suit its overall needs and endowed with faculties and powers far beyond those of its constituent parts" seemed too neat and too simple. But as the microbial world reveals more and more of its secrets, this idea of global homeostasis, of a planetary balancing act, grows more and more intriguing. Maybe it is still far fetched. Or maybe microbes, if we give them half a chance, can play a key role in helping the world's ecosystem keep its biological footing. Read and decide for yourself.

— Erica Goldman

***The long goodbye — gone are the bowling lanes and dry cleaners** that once drew customers to this strip mall north of Baltimore, but below ground a soup of toxic chemicals lingers. Will naturally occurring bacteria break down these persistent compounds, or will this become another toxic legacy in the Chesapeake watershed?*

PHOTOGRAPHS BY JOEL KLEIN, EXCEPT STREAM, BY ERICA GOLDMAN.

Of Microbes & Messes

Bacteria Hold Key to Cleaning Up Polluted Groundwater

By Erica Goldman

Its red letters are now faded and rusty, but the old "BOWL" sign still towers above an abandoned parking lot in Towson, Maryland, a reminder of a time when teenagers flocked to Fair Lanes and customers brought their rumpled shirts and slacks to Kings Cleaners, a dry cleaning operation that stood next door. Now the bowling alley is a roofless cement hull. Yellow and blue paint peels off the sides, rows of steel girders rust beneath the sky. In the old bowling alley's vast interior, gleaming new construction equipment rests idle — the only sign that the site will soon be redeveloped.

Deep below the paved lot surrounding the abandoned bowling alley, and well out of sight, a contaminated groundwater plume slowly creeps toward Mine Bank Run, a tributary of Gunpowder Falls. Kings Cleaners has vanished entirely — except for a square gravel stain in the center of the parking lot. But its chemical signature lingers strong and clear. Twenty feet beneath the former dry cleaning operation, tetrachloroethene (PCE), a widely used cleaning solvent, persists in the groundwater at high concentrations, measured at 16,400 parts per billion (ppb) in 2004, more than 3,000 times the legal limit of 5 ppb set by the U.S. Environmental Protection Agency.

PCE is a suspected human carcinogen and difficult to clean up. It can contaminate groundwater, a grave concern in rural areas that rely on wells for their drinking water supply. When this compound percolates close to the surface along with groundwater, it can intrude into the soil, releasing volatile, noxious gases. Short-term PCE exposure can cause dizziness, headaches, and problems with balance. Over the long term, PCE exposure has been linked to cancers of the esophagus, bladder, and blood.

Kings Cleaners closed in 2000, after continuous operation for 32 years. In 1998, the Maryland Department of the Environment (MDE) began a series of environmental assessments that lasted for years — testing contaminant levels in the groundwater, in soil gases, and in nearby residential wells — to determine whether remediation would be necessary prior to any future development.

In October 2005, MDE decided to allow limited industrial and commercial development at the site, as long as the groundwater remains untouched. Tests had deemed the soil gases free of PCE. So the chemical poses no immediate threat to future occupants of the site. The agency issued what is known as a "No Further Requirements Determination" to the current property owner, meaning that no remediation steps would be mandated. But no residences can ever be constructed on the site. No wells can ever be dug.

Sites like the former Kings Cleaners exist all over the United States. According to a study by the State Coalition for Remediation of Dry Cleaners, 75 percent of all active dry cleaning facilities are contaminated (some 17,000 sites nationwide), and that doesn't include sites that have been abandoned.

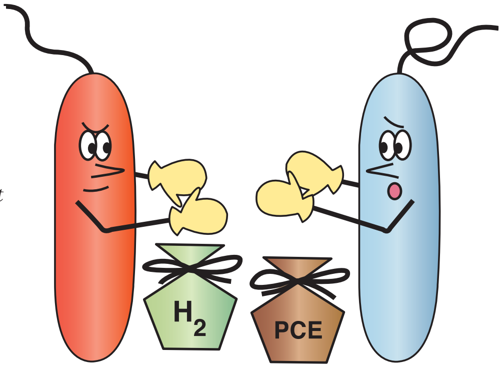
Are there tools to clean up these toxic messes? Scientists know that there is one species of bacteria — only one — that can break down PCE and its chemical cousin TCE (trichloroethylene), a compound with a similar chemical structure used in metal degreasing and paint stripping. To this "bug" (*Dehalococcoides ethenogenes*), chlorinated solvents such as PCE and TCE are the air that they breathe. So shouldn't cleaning up PCE-contaminated sites be as simple as providing this toxin-breathing bug unfettered access?

It's not that easy. In nature, a single species of bacteria rarely acts alone. Microbes live in intricate consortia and interact with other organisms — in the marine environment these include algae, corals, and sponges. Different bugs may play complementary roles, or they may compete fiercely. Scientists have long worked to harness the power of microbes to clean up human messes. Now, more and more, they are looking to the ecology of bacteria for clues about how to tackle tough challenges — like erasing the environmental signatures of industrial pollution.

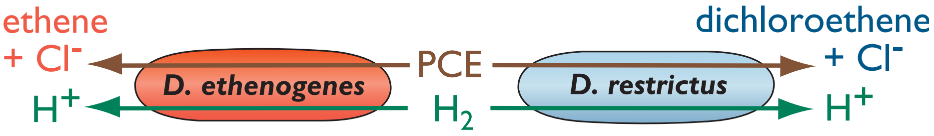
Fierce Competitors

Jennifer Becker leaves her office in the Biological Resources Engineering Department at the University of Maryland in College Park and walks briskly across campus to the Plant Sciences Building to give an invited lunchtime seminar. She sets up her computer and plugs into the room's audio-visual system as the previous class files out.

Her first Power Point slide features two potato-sized shapes, thousands, maybe millions of times the size of the bacteria they represent. Their stick figure arms wear boxing gloves. Facing each other, they square off, ready to duke it out.



***In fierce competition for hydrogen,** the bug* Dehalococcoides ethenogenes *squares off against* Dehalobacter restrictus*, another chlorine-breathing microbe. Scientist Jennifer Becker uses this drawing to explain her work to students and colleagues. Both bugs need hydrogen for cellular respiration. But fans should root for* D. ethenogenes *(red boxer), the only bug that can use hydrogen to render PCE, a toxic chemical used by the dry cleaning industry, completely harmless as ethene and chlorine (shown below in another of Becker's drawings).*



Microbes operate in complex environments, she explains as she clicks the remote. If, she says, our goal is to clean up human-made messes, like chlorinated solvents from the dry cleaning industry (PCE), we need to understand not only how these bugs function metabolically, but how they interact with each other.

Becker's schematic duel depicts a simulated competition between two different species of bacteria. One bug (*D. ethenogenes*) can render toxic chemicals totally harmless. The other bug can only go halfway, leaving a still nasty chemical compound (dichloroethene) in its respiratory wake.

This microbial duel showcases a classic battle for a limited resource. In this case the bacteria fight over hydrogen, a microscopic version of two carnivores fighting over a ravaged wildebeest in the African Serengeti. Hydrogen, explains Becker, is especially important because it acts as an electron donor, providing the engine for cellular respiration. But other bacteria also rely on hydrogen to power their metabolic needs and some can use it faster than *D. ethenogenes*, beating it to the punch. In these cases, competition may not favor the complete breakdown of the toxic compounds if the faster microbe only does half the job.

The trick, she explains, is to engineer the situation so competitors do not overwhelm the more thorough bug, the one that can take the chemical completely out of commission.

Becker's pictorial representation frames a conceptual model she's developed to simulate the effects of competition among bacteria in the environment — a model that revamps traditional thinking. The current paradigm in the bioremediation industry for PCE cleanup is simply to determine whether the best bug (*D. ethenogenes*) is present in the subsurface aquifer, explains Becker. If it is not, consultants will recommend that you add it. According to one count, such projects to clean up groundwater contamination related to industrial dry cleaning operations have already been implemented at 17 sites in ten states.

"But I've shown through modeling that competition in the environment could cause the added bug to be lost," she says. If we know that different bugs are competing for a limited amount of hydrogen, she explains, we may have to develop new strategies to ensure that both organisms get the hydrogen and other resources that they need. Becker describes her modeling work in the July 15 issue of the journal *Environmental Science & Technology*.

Michael W. Fincham

Michael W. Fincham

Electron Microscope Laboratory, Berkeley, CA

Looking for Mr. Goodbug — *Jennifer Becker and her student Yen-jung Lai check out a bacteria called* D. ethenogenes *(shown in micrograph, above, and glass bottle, above right). This microbe breathes a chemical called PCE, short for tetrachloroethene, a widely used, long-lived cleaning solvent which fouls groundwater under thousands of industrial sites.*

As Becker continues her talk, the audience listens intently, trying to absorb detailed information about how *D. ethenogenes* breaks down chlorinated compounds like PCE and TCE. As she concludes, she's got time to take a few questions from the audience before the next class takes over the lecture hall. One colleague asks whether she knows if *D. ethenogenes* is widely distributed (or ubiquitous) in nature. This is one of the big unknowns, Becker responds, and something that she is working to find out.

In the past few years, molecular biology tools have made it easier to determine whether these bacteria are present and active in the environment, she says. But these tools often cannot provide information about how the bacteria function in the environment. In order to validate this model of competition in the environment, we must grow the bacteria in pure culture, effectively staging the competition, and see which one wins out, she explains.

One of her graduate students is also working on painstaking kinetic rate measurements, to determine how fast these bacteria carry out certain reactions, which will also help to ground-truth Becker's theory.

"Eventually, we want to be able to go back to the environment and make predictions about whether we would expect to find one bug or the other at a particular location," she says. In the future, Becker plans to test her model predictions at a site where there's a contaminated groundwater plume.

Balancing Act

As Becker leaves the lecture hall to walk back to her office, she doesn't linger long in the afternoon sunshine. She's in a hurry to meet her students in the lab to help them troubleshoot a problematic protocol. Focused and directed, she makes each second of her day count.

Becker's focus keeps her grounded in a challenging career juggling act. A young assistant professor with a big lab, Becker also holds an appointment in Maryland Cooperative Extension as an extension specialist in waste management. She focuses on education and outreach in the community — "anything with a treatment process," ranging from animal manure to stormwater treatment to bioremediation. Each year, she also leads sessions in a job-training program in Baltimore, preparing individuals for entry-level jobs in environmental fields.

In any given week, Becker's days range from academic work based in the lab to applied practical work with citizens of the state. She's involved in a lot of traditional agricultural engineering extension, teaching farmers how to manage manure so "nutrients don't go where they're not supposed to go." This ties her work directly to the challenging issue of slowing the flow of nutrients to the Chesapeake Bay.

She's also been involved in recent efforts to educate farmers about changes that affect implementation of federal air quality regulations. A national study to monitor emissions from animal feeding operations seeks to set size limits for animal operations defined by their emission output — for example, a poultry operation with over 75,000 chickens might be determined to emit too much ammonia, explains Becker. Animal operations that exceed size-based thresholds will be considered emitters and subject to potential lawsuits under a violation of the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). Becker works to keep citizens aware of what these changes could mean for their businesses.

"Finding a balance between my research and extension programs has been huge," says Becker. "Sometimes I do feel spread thin. But it is very rewarding to help farmers do their job better and to help people get jobs. That is very inspiring to me," she says.

Becker's balancing act seems to be working. In 2002, she received a prestigious Faculty Early Career Development Award (CAREER) from the National Science Foundation (NSF). NSF subsequently selected her from among CAREER grant awardees to receive a Presidential Early Career Award for Scientists and Engineers. This award, established by President Clinton in 1996, is considered to be "the highest honor bestowed by the United States government on scientists and engineers beginning their independent careers."

Pollution Evolution

After a quick stop by her office, Becker heads down to the lab to work with her students on their troublesome protocol. She grabs a can of seltzer on the way down, but doesn't break for lunch. Her post-doc, Hong Yin, and student, Yen-jung Lai, are working with growth media used to maintain a large volume of

Wanted: Cleans Up PCBs, No O_2 Required

Baltimore Harbor is an anaerobic microbiologist's dream. "The sediments are black and oily, almost like black Jell-o," says Kevin Sowers, a scientist at the University of Maryland Biotechnology Institute's Center of Marine Biotechnology.

Sowers studies anaerobes — bacteria in the sediment that thrive without oxygen. "Lots of people don't think about the anaerobes," he says. "People assume that there is oxygen in the world and that living things need it." In reality, Sowers says, probably half the microbes out there are anaerobes.

Sowers's particular passion: anaerobes that can break down polychlorinated biphenyls (PCBs), chlorinated compounds used heavily by the manufacturing industry. PCBs were banned in 1977 by the U.S. Environmental Protection Agency (EPA) because of their link to certain cancers, liver, and skin problems. Although similar in structure to the PCE and TCE studied by Jennifer Becker (see Of Microbes and Messes, p. 4), PCBs do not linger in the groundwater. Instead they stick to sediment in rivers and streams and slowly work their way up the food chain — passing from worm to fish to bird — persisting for decades or more.

Tributaries with a history of industrial and shipping activities, such as the Patapsco and Anacostia Rivers, have contributed significantly to PCB contamination in the Chesapeake Bay. White perch from more than 20 of the estuary's river systems harbor PCBs in their body tissue, according to EPA's Chesapeake Bay Program, which monitors these fish as indicators of contaminant levels. In Baltimore's Patapsco River, levels of the compound in white perch exceed the zero meals-per-year advisory established by the state, which means that these fish should not be consumed at all.


Erica Goldman

After the EPA banned the use of PCBs, companies that discharged these compounds into waterways suddenly faced the prospect of cleaning them up. General Electric, which operated manufacturing plants on the Hudson River for roughly 30 years (1947-1977), bore responsibility for contaminating 200 miles of the Hudson River Estuary in New York — the removal of a dam had flushed 1.3 million cubic yards of PCB-laden sediment downstream. The company invested in research, spearheading an effort to prove that microbes would take care of the problem on their own.

But soon scientists realized that while the sediment seemed to contain some anaerobic bacteria that could break down PCBs, the process appeared to unfold very slowly and often not to an endpoint that completely detoxified the chemical. And although they had identified groups of bacteria (consortia) collectively responsible for degrading PCBs, they had not been able to catch the specific bugs in action — making the development of technologies for microbial bioremediation impossible. The EPA determined that dredging would be the only acceptable option for cleaning up PCBs. Today General Electric and the EPA are poised to begin a large-scale dredging operation in the Hudson River to the tune of 2.65 million cubic yards of sediment.

But "you can't dredge the entire Eastern coastline," says Sowers. "It will take a combination of technologies." Sowers has built his scientific career trying to understand anaerobes — especially those unique microbes that use chlorine atoms to breathe, don't need oxygen, and can take apart stubbornly recalcitrant PCBs. His persistence and patience have finally paid off.

Sowers's preliminary efforts in Baltimore Harbor demonstrated that conventional microbiological techniques would not be sufficient to identify the specific microbe that could degrade PCBs. He suspected that part of the difficulty stemmed from the fact that bacterial culture. The media shows signs of contamination. They've recently seen white filamentous organisms growing in it that shouldn't be there. With Becker supervising, they plan to work through the preparation steps to see if together they can identify the problem.

As they prepare their laboratory equipment, Becker shifts mental gears and enters her practical problem-solving mode. She knows that her ability to prove her theory about how bacteria like *D. ethenogenes* compete in the environment depends on the success of these time-consuming experiments, which require meticulous attention to detail.

Nature may not always provide a convenient means of cleaning up human messes.

But Becker never loses sight of the bigger context for her research. She's propelled by the idea that the better we can understand how these PCE and TCE-breathing bacteria do what they do, the better we will become at harnessing their power to clean up contaminated sites.

She recognizes that her work on how pollutant-busting bacteria function in their natural environment touches on a question that is even bigger still. How did these bacteria "learn" to break down compounds that they've never encountered before?

The environment has only seen compounds like PCE and TCE for a short time, since after World War II, explains Becker. But bacteria that can break down these compounds did not appear out of

See Microbes, page 12

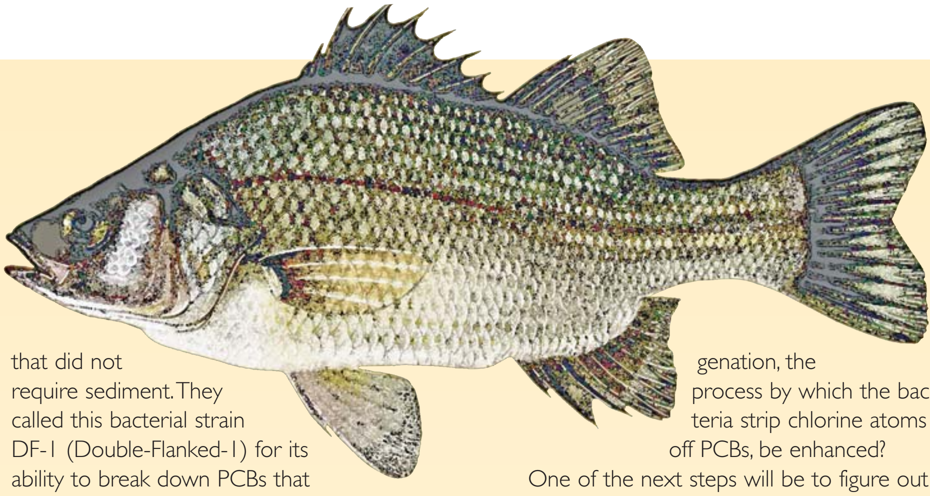
Erica Goldman

To study PCB-breathing microbes *that thrive in environments with no oxygen, UMBI microbiologist Kevin Sowers (opposite page) uses an apparatus called a glove box (above), which creates an oxygen-free workspace. White perch (above right) serve as biological indicators of PCB contamination in the Chesapeake Bay food chain.* DRAWING BY DIANE ROME PEEBLES.

other bacteria outnumbered the PCB-breathers (dehalogenators) in his samples.

Sowers used molecular probes to detect the bacteria's presence in his sediment samples, in collaboration with colleague Harold May, a microbiologist at the Medical University of South Carolina in Charleston who worked extensively in the Hudson River system. At first they found that the PCB-breather seemed to depend on the sediment to thrive. When Sowers and May removed the sediment, the microbe's activity would die out. After time, however, they could again measure microbial activity with molecular techniques. As the bacteria began to adapt to life without sediment, their activity grew stronger and stronger.

Nine months later, in 1996, Sowers and May finally isolated chlorine-breathing bacteria that did not require sediment. They called this bacterial strain DF-1 (Double-Flanked-1) for its ability to break down PCBs that have chlorines flanked by other chlorine atoms. This bug proved so small that even a fluorescent microscope could not detect it.



Now Sowers and his colleagues are beginning to find out what makes DF-1 tick. They know that DF-1 associates closely with the microbes in the genus *Vibrio*, bacteria that are very common in the marine environment. Understanding the nature of this association with *Vibrio* may prove critical. Sowers suspects that *Vibrio* may provide either nutrients or electrons to DF-1, although the basis of the association is not obvious. Sowers is now working to figure out what *Vibrio* supply to DF-1, information that will likely prove critical in putting DF-1 to work cleaning up PCBs (for more information about microbial associations, see "Mutual Arrangements," p. 10).

Technologically speaking, the potential to use microbes such as DF-1 to clean up PCBs is still a long way off. But with the identity of the bug in hand, Sowers and his colleagues can now craft a specific molecular probe to detect its presence in the environment. They can begin to ask the important questions. Where are these bacteria naturally present? Do they aggregate only in PCB-contaminated areas or can they thrive elsewhere? How can dehalogenation, the process by which the bacteria strip chlorine atoms off PCBs, be enhanced?

One of the next steps will be to figure out what physical factors in the environment affect the ability of this newly isolated microbe (DF-1) to degrade PCBs.

PCBs tend to attach tenaciously to activated carbon, a dry, granular substance that can remove organic substances from water. If one could till large amounts of activated carbon into PCB-contaminated sediments, the PCBs would be effectively locked up and blocked from making their way up the food chain. But how long will the PCBs remain bound to the carbon? And will DF-1 still be able to degrade the contaminant if it is bound?

Sowers is working to answer these questions, in collaboration with Upal Ghosh, an environmental engineer at the University of Maryland Baltimore County, and chemist Joel Baker at the Chesapeake Biological Laboratory, part of the University of Maryland Center for Environmental Science. This research, funded by the Department of Defense, offers one promising remediation strategy for PCBs in the marine environment.

For now, dredging may remain the only remediation option for PCBs accepted by the EPA. But Sowers and his colleagues are working hard to change the status quo.

— E.G.

Mutual Arrangements

Small-scale Symbiosis of the Seas

© Wolcott Henry 2005

> *Microbes enter into collaborative concords that make them far more potent than they might be alone.*

Bacteria live ecologically complicated lives. They inhabit some of the most extreme environments on earth and can break down some of the most noxious compounds known. They compete with each other for limited resources (see "Of Microbes and Messes," p. 4). But they also enter into collaborative concords, intricate symbioses with other organisms that make them far more potent than they might be alone.

Like members of the visible world, microbes can enter into mutually beneficial treaties with other organisms. Picture the clownfish that darts unscathed around a sea anemone's poisonous tentacles or the Egyptian plover that rides astride a crocodile, freeing it from parasites while reaping a ready source of food. Now picture these relationships unfolding on a scale many orders of magnitude smaller than the eye can see.

When the infamous marine dinoflagellate *Pfiesteria piscicida* roared into the headlines in 1997, with reports that it released a fish-killing toxin in the Chesapeake Bay, microbiologist Robert Belas went to work to identify the different bacteria associated with *Pfiesteria*. His hypothesis: microbes that clustered around this dinoflagellate might play a role in its suspected "toxicity." Understanding the nature of these novel symbioses would be the first step toward validating this idea.

As part of a team of scientists from the University of Maryland Biotechnology Institute's Center of Marine Biotechnology in Baltimore, Belas readily found that the notorious dinoflagellate consorts with an impressive group of followers, over 30 different species associated by some sort of symbiotic link. In a story of discovery that would unfold over the next decade, Belas embarked on a journey that would lead him from *Pfiesteria* to oceanic sulfur metabolism to coral bleaching, ultimately steering him towards possible links between bacteria and rising global temperatures.

Belas soon found that sulfur was the great common denominator between the associated bacteria and *Pfiesteria*. He discovered that a group of bacteria called *Roseobacter* dominated his cultures and that this group uses sulfur (by oxidation) to make its nutritional way in the world.

Wondering why sulfur seemed to link these bacteria to *Pfiesteria*, Belas and his graduate students found themselves drawn deeper into the basic question of what made this association tick. For the time being, the path led away from the immediate question of *Pfiesteria*'s toxicity and into the mysterious world of oceanic sulfur metabolism.

Todd Miller, Belas's graduate student, began to focus on an organic sulfur compound known as DMSP (dimethylsulfoniopropionate), one of the most abundant sources of sulfur in the marine environment. Since many unicellar dinoflagellates and algae make DMSP, Miller realized that the next logical step would be to determine if *Pfiesteria* also makes the sulfur compound.

When Miller found that *Pfiesteria* does in fact make DMSP, it seemed likely that some of these sulfur-oxidizing bacteria might be drawn to *Pfiesteria* because of the sulfur compound. Miller homed in on one of these sulfur-oxidizing microbes, classified it, and named it. He called the bug *Silicibacter* sp. TM1040 (for Todd Miller).

The connection between TM1040 and *Pfiesteria* turns out to be a tight one. TM1040 has sensory mechanisms to detect DMSP in the surrounding water. It can swim toward the source (*Pfiesteria*), and firmly attach itself to the dinoflagellate. This close physical association, explains Belas, suggests an evolutionary adaptation on the part of the bacteria to get close to their source of nutrition.

TM1040 also seems to have evolved a trick to keep competitors away from it, says Belas. With the help of an undergraduate stu-

Erica Goldman

Following a long trail *from* Pfiesteria *to the open ocean, UMBI microbiologist Robert Belas (above) has tracked down the ways in which bacteria may use antibiotics to protect a range of hosts, including corals. Corals that lose their symbiotic algae (zooxanthallae) look ghostly white or "bleached" (shown on opposite page).*

dent supported by Maryland Sea Grant, Belas's group found that TM1040 produces an antibacterial compound (an antibiotic) that inhibits pathogens such as *Mycobacterium marinum*, *Vibrio anguillarum*, and *Vibrio cholerae*, which can affect fish and algae.

Does antibiotic protection hold the key to the close association between *Pfiesteria* and TM1040? Maybe, says Belas. The bacteria (TM1040) seem to benefit *Pfiesteria* with their antibiotic activity by keeping pathogens away and in turn receive open access to a source of carbon and sulfur. But this work is still preliminary, he cautions. The research team has not yet shown that the antibiotic is even produced when the bacteria is in contact with *Pfiesteria*. The group is working to develop an assay to measure gene expression in order to test this concept, he says.

Commercially, TM1040's antibiotic could be developed for aquaculture purposes, to add to the water as a probiotic to protect fish from diseases such as *Mycobacterium*, says Belas. But antibiotic production by TM1040 and related bacteria may have even broader implications, he says, for the health of the seas.

Coral Connection

The antibiotic-producing prowess of TM1040 could have effects that reach far beyond local waters. Antibiotic production by bacteria like TM1040 may protect corals from bleaching and counteract some of the effects of rising global temperatures, says Belas.

Bacteria similar to the TM1040 live in the mucus that surrounds corals. And the antibiotic produced by TM1040, Belas found, can kill two of the pathogens connected with coral bleaching (*Vibrio shiloi* and *Vibrio coralliilyticus*).

Again sulfur seems to play a key role in the story. Corals harbor algal cells inside their body (called zooxanthallae) that in turn depend on the coral for survival — a symbiosis of clear co-dependence. Like *Pfiesteria*, these algal cells also produce the sulfur compound DMSP. If the relationship between these TM1040-like bacteria and corals proves similar to the one between TM1040 and *Pfiesteria*, sulfur would be the carrot that draws the bacteria close.

But a link is still missing. Belas suspects that TM1040-like bacteria may keep the coral healthy by producing antibiotic compounds that protect them from other pathogens. He does not yet know whether TM1040 itself lives in the mucus surrounding corals, only that similar species do.

If bacteria do provide antibiotic protection to keep corals healthy, can they still thrive if the world's water temperatures rise?

All microbes have an optimum temperature range at which they function best. TM1040 is no exception. It stops growing

See Symbiosis, page 12

Medicine from Microbes

Russell Hill

From the exotic waters *of Indonesia, come microbe-rich sponges like this one (*Xestospongia testudiniaria*). UMBI microbiologist Russell Hill scours these sponges for bacteria that can produce compounds promising for medicine.*

Symbiotic relationships between bacteria and other marine creatures may prove a treasure trove for drugs from the sea. Bacteria associated with sponges and sea slugs may harbor new treatments for malaria and cancer.

Russell Hill, a marine microbiologist at the University of Maryland Biotechnology Institute's Center of Marine Biotechnology, has discovered two novel bacterial symbionts. One lives inside an Indonesian sponge and produces a compound with anti-malarial properties; the other lives inside a small sea slug (called a sacoglossan) and produces a peptide with promising anti-cancer activity, currently in Phase II clinical trials in Europe.

The key is to divorce the drug-producing microbe from its sometimes-scarce host. This may make all the difference for drug development, says Hill. Growing the bacteria in the lab is cheap relative to finding and harvesting it from the animal itself, and lab culture offers the opportunity to make genetic modifications once the gene that encodes for the compound of interest is isolated.

"The problem is supply," he explains. Many companies receive licenses for compounds from the sea but stall when they realize that they can't get enough from harvesting whole macro-organisms, like sea slugs or sponges. Especially for a disease like malaria, the drug company can't afford to spend that much money making the compound because they need to keep the cost of the drug low. They need a more economical source.

"There are lots of exciting compounds out there, but few have made it to market," Hill continues. In fact, only a single drug of oceanic origin has been approved by the Food and Drug Administration — a small peptide from a cone shell mollusk that can be used to treat severe pain.

Hill's success in isolating these two drug-producing microbes from their host, one of the first times this has been accomplished, may open a new chapter in development of drugs from the deep. Working in constant collaboration with Mark Hamann, a marine natural products chemist from the University of Mississippi, he continues to look towards bacteria as the pharmaceutical jackpot of the undersea world. He's focusing closely on sponges now, which are filled with bacterial cells — in some cases 50 percent of their weight comes from their microbial inhabitants. Many sponges have over 200-300 different compounds associated with them, mainly to prevent algae from growing or fish from feeding on them.

Most of the symbionts in sponges are novel and have not yet been grown in culture. For the most part, no one yet knows which symbionts are producing which compounds or even which compounds might have important pharmaceutical powers. But Hill is on the case.

— E.G.

Symbiosis, *from page 11*

at a temperature of 32°C and stops producing antibiotic at 29°C, says Belas. Coincidentally, most coral bleaching occurs when waters rise to 30°C or above. Preliminary evidence also suggests that bleached corals do not have TM1040-like organisms as part of their normal mucus flora and will often have pathogenic *Vibrio* in their place.

Following this train of logic, rising water temperatures may thwart the ability of the bacteria to make antibiotic, which makes corals vulnerable to bleaching. "I have no data to support this yet," cautions Belas. "It is entirely coincident."

But Belas is not alone in his thinking. Additional support for the idea that antibiotics may help prevent coral bleaching comes from Kim Ritchie, head of the Coral Microbiology Program at Mote Marine Laboratory in Sarasota, Florida. She's found that when water temperatures are cool, corals have a tremendous diversity of bacteria in their associated mucus, including some that have the ability to produce antibiotics. Bleached corals in warmer waters lose this bacterial diversity and tend to harbor an overabundance of pathogenic *Vibrio* in their place.

"It's a good hypothesis," says Ritchie of Belas's suggestion, "but it will be difficult to prove." Just because bacteria produce antibiotics in the lab, does not mean that they produce them in nature, she explains. "There is so much going on in communities of organisms associated with the coral's symbiotic algae (zooxanthallae)," Ritchie says.

The emerging picture involving microbes, coral mucus, and temperature change is complex, agrees Garriet Smith, a marine microbiologist at the University of South Carolina. "Temperature changing over time affects everything, the zooxanthallae, the biota, interactions with bigger organisms," says Smith. "Any change that weakens one component and strengthens another is potentially important."

What started as a quest to figure out the source of *Pfiesteria*'s suspected toxicity has become part of a much bigger story that links the smallest of organisms (bacteria) to the largest of Earth's problems (rising global temperatures). Today, the *Pfiesteria* conundrum remains an open question. But Belas's work promises to recalibrate our perception about the scales over which different organisms connect to each other. With each new discovery, we come closer to understanding the complex role of tiny microbes in a global context, closer maybe, to exploiting their talents to preserve the delicate balance of the global ecosystem.

— E.G.

Microbes, *from page 9*

nowhere. "*Dehalococcoides* did not magically evolve these enzymes overnight," agrees Rekha Seshadri, a microbiologist at the Institute for Genomic Research in Rockville, Maryland.

For centuries, bacteria have had natural compounds similar to PCE to work on, explains Becker. However, their concentrations are much lower than can be found at contaminated sites. Some insects, for example, produce chlorinated compounds to protect themselves in what could be considered chemical warfare, and volcanoes may also produce related compounds, Becker says.

Bacteria that break down these natural PCE-like compounds likely acquired mutations that allowed them to respire the man-made ones. Then as pollution in the environment became more widespread, bacteria that could live off these new toxins thrived, Seshadri says, thus selecting for bigger and bigger populations of these bacteria.

Seshadri spearheaded the efforts of a group of scientists to sequence the genome of *D. ethenogenes*, which was published in *Science* last year. The bug's genetic code revealed its complete dedication to the process of breaking down compounds like PCE. Its genes contained very little extraneous information — only the bare bones necessary to encode the enzymes directly involved in the breakdown process.

The microbial goings-on beneath the former Kings Cleaners remain a mystery.

The genome sequence also provided hints that *D. ethenogenes* is evolving rapidly and customizing its physiology to meet the demands of the environment, explains Seshadri. The group found that structures called "integrated islands" make up a surprisingly large percentage of the bug's total genetic material. These "islands" are like footprints of a recent genetic exchange. They reveal areas where *D. ethenogenes* may have swapped material with another species of bacteria.

Many bacteria like *D. ethenogenes* appear to evolve rapidly to meet the demands of new and increasingly obnoxious chemicals in the environment —

Erica Goldman

Change comes to the human landscape, *as the crumbling shell of an abandoned bowling alley makes way for a new warehouse. State officials are certifying that the soil is safe, but not the groundwater below. No wells will be allowed on this location. The subterranean aquifer must remain undisturbed.*

acting as invisible checks and balances on the forces pulling the Earth away from equilibrium. Nature may not always provide a convenient means of cleaning up human messes, says Seshadri. And in the meantime, such pollutants continue to take their toll on human life and health.

Ground Truth

Becker finishes with her students in the lab. She has not definitively pinpointed the source of contamination, but she has identified some other problems with their protocol. For example, she notices that the iron solution that is added to the culture has turned orange, indicating that the solution was prematurely exposed to air. Iron helps sop up oxygen, which is harmful to the bacteria, explains Becker. Since this solution had already been in contact with air, it would not be effective at scavenging oxygen in the culture media.

"This is why it is good to get down to the lab," she says.

Becker takes her job as graduate mentor seriously. She has five graduate students and a post-doctoral fellow, and she meets with each one individually every Friday. She helps them to evaluate their work from the previous week and set goals for the next one. By the end of each Friday, her head is spinning but she feels that these meetings really help her students stay on track.

Every other week, her lab meets as a group to hear a presentation from one of its members. Now Becker hurries upstairs from the lab to a classroom to listen to one of her Ph.D. students, Deyang Huang, talk about his recent experiments. Huang is studying how fast another PCE-breather (*Desulfitobacterium* sp. strain PCE1) can carry out certain reactions. Unlike *D. ethenogenes*, strain PCE1 cannot completely detoxify PCE. This bug instead converts PCE to the related compound TCE. Bacteria such as strain PCE1 thrive at contaminated sites and can also contribute to the removal of PCE from groundwater, Becker explains. But these bugs may also divert resources, including PCE, away from other potentially important organisms like *D. ethenogenes*.

Huang's results leave Becker a little puzzled. The reaction rates that he measured in these so-called kinetic experiments do not seem to match the values that she has extrapolated from the scientific literature for her mathematical model. His data also show that the rate at which the bug breathes and metabolizes PCE depends on the age of the source culture, a likely indication that the culture had not achieved the desired "steady state" before he began the experiment. Although Huang's data are not conclusive, Becker expects that the experiment, which took months to complete, will need to be repeated before the results can be interpreted.

Meanwhile in a subterranean aquifer beneath former Kings Cleaners in Towson, roughly 60 miles north of Becker's lab, PCE persists in the groundwater at high concentrations, even as changes begin to unfold aboveground. Soon ABC Rentals, a business that rents tools and hardware, will build a warehouse on the part of the site where the bowling alley once stood. The footprint of the dry cleaners remains unclaimed as of yet, but several companies have placed bids for the space, says Debbie Haney, the broker handling the property for Mackenzie Commercial Real Estate Services.

The microbial goings-on beneath the former Kings Cleaners remain a mystery. Perhaps *D. ethenogenes* is already hard at work underground, respiring the toxin PCE and rendering it harmless. Or maybe the PCE-breather is nowhere to be found in this groundwater. Or perhaps other bacteria have beaten it to the punch, stealing the necessary resources and depriving it of the opportunity to work its metabolic magic. With each experiment, Becker's research unlocks new clues to how these microbes behave on their home turf, clues that will ultimately improve our ability to readily clean up contaminated groundwater. Her research findings will come too late to change the course of action for the site on East Joppa Road in Towson. But plenty of PCE-contaminated sites all over the country still need to be cleaned up. A clearer understanding of microbial competition promises to help the bioremediation industry encourage bacteria to do what they do — even better.

For More Information

Jennifer Becker and PCE

Becker Lab Home Page
www.bre.umd.edu/becker.htm
PCE Factsheet from the U.S. Environmental Protection Agency
www.epa.gov/safewater/contaminants/dw_contamfs/tetrachl.html
TCE Factsheet from the U.S. Environmental Protection Agency
www.epa.gov/safewater/contaminants/dw_contamfs/trichlor.html
State Coalition for Remediation of Drycleaners
www.drycleancoalition.org/

Kevin Sowers and PCBs

Kevin Sowers Lab
www.umbi.umd.edu/~sowers/home.html
EPA site on Hudson River dredging
www.epa.gov/hudson/
Chesapeake Bay Program Indicators
www.chesapeakebay.net/status/status_dev.cfm?SID=206&SUBJECTAREA=INDICATORS
EPA Indicators for Chesapeake Bay Health
www.epa.gov/maia/html/cbhealth.html
Marinebiotech.org
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Robert Belas and Microbes

Robert Belas's Lab
www.umbi.umd.edu/~belas/
Marinebiotech.org
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Russell Hill and Medicines

Russell Hill's Lab
www.umbi.umd.edu/~comb/faculty/hill/hill.html
Marinebiotech.org
www.marinebiotech.org/hill.html

Ronald Weiner and NSF

Research Profile
www.life.umd.edu/grad/mocb/faculty/wiener.html
National Science Foundation Cellular Systems Cluster
www.nsf.gov/funding/pgm_summ.jsp?pims_id= 12772

Profile

Teasing Out Microbial Secrets

By Erica Goldman

Sandy Rodgers

When Ronald Weiner logged his 10,000th grade assignment in 2001, he decided that it was time to shift the emphasis of his career. For more than 30 years, Weiner has taught microbiology to students at the University of Maryland, College Park (UMCP) with passion and humor. At times Weiner's teaching assistant would introduce him in the spirit of Johnny Carson. "Here's Ronny…" would bring Weiner to the front of the lecture hall and he'd start each class with a brief monologue, jokes that he hoped were relevant to the subject at hand. "My first love has and will always be teaching," he says, but it was time, he decided, to focus on bringing closure to his prolific academic career.

Weiner's love for science rivals his zeal for teaching. For as long as he can remember, he has been interested in the world beyond the reaches of human perception. He was the first kid on his block with a microscope, the first to jab his finger and look at his blood up close.

Born from this youthful curiosity about the natural world, Weiner's career in science has led him down twisting paths to some important and unexpected discoveries. During his tenure at UMCP, he's made major contributions in two areas within microbiology. He's helped to unveil the biochemical secrets of mysterious microbial communities that create biofilms on boat bottoms and oyster bars. He's also found a species of bacteria that breaks down complex carbohydrates in the ocean, a discovery that helps to fill a key gap in our understanding of the marine carbon cycle. Of his journey, Weiner says, "It has been a good ride."

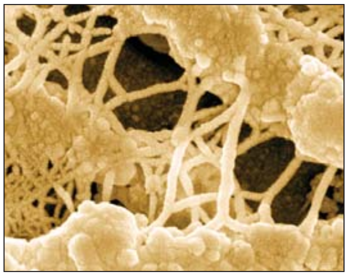
Forming Films

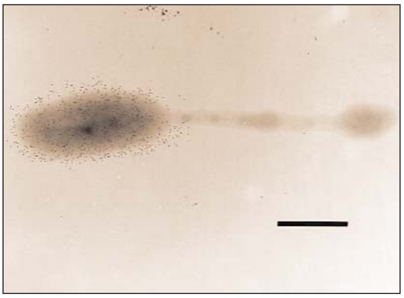
When Weiner first joined the faculty in Microbiology at UMCP in 1970, his scientific efforts focused on studying how cells differentiate and age, one of the fundamental questions in microbiology.

As a model for cellular aging, he used the bacteria *Hyphomonas,* whose two-phase life cycle made it ideal for studying life cycle transitions. But this microbe is also a primary colonizer in the marine environment, a fact that soon sent Weiner riding down an unanticipated road. *Hyphomonas* is one of the first organisms to form complex biofilms that stick to and foul the bottoms of ships, providing a fertile home for many marine creatures, a fact that drives boat owners to expensive haul outs for scraping and repainting jobs nearly every year. The U.S. Navy, owners of big boats like battleships and aircraft carriers, wanted a better understanding of ways to control biofilm buildup. Weiner soon had a new program underway, thanks to support from the Office of Naval Research.

Before long he made some unforeseen discoveries that had nothing to do with boats and a lot to do with oysters. Biofilms, as it turns out, strongly attract oyster larvae floating in the water and encourage them to circle down and settle into place. With support from Maryland Sea Grant, Weiner explored the biochemical basis of this attraction, identifying the compounds produced by the biofilm's bacteria that induce oyster larvae to initiate stereotypic swimming patterns. "The larvae would detect [these compounds], swim in a circle like a dog circling a fire hydrant, always in the same direction, and then settle down," Weiner describes.

From cellular aging to biofilms to oysters — out of this fundamental research, Weiner and his collaborators dug up some very practical applications. Weiner's oyster findings spawned the St. Georges Oyster Company, a commercial oyster hatchery down in St. Mary's County. The company validated Weiner's ideas that manipulating biochemical cues inducing larval settlement could increase oyster yield. A second company — the aptly named Adheron — seized on his findings about the adhesive power of





***During a long and distinguished career,** Ronald Weiner has followed the path of two enigmatic microbes.* Hyphomonas adherans *(bottom left), a primary colonizer in microbial communities called biofilms, produces a sticky adhesive that allows it to adhere to surfaces with great force. The other,* Sacchariphagus degradans 2-40 *(top), is a degrader-extraordinaire. It can break down hard-to-digest marine material, such as algae and chitin, and make underwater plants disappear, as shown in the flask above.*

Photographs by Ronald Weiner

biofilms. "If a bacterium [like *Hyphomonas*] can glue itself next to a ship's propeller in a turbid environment, that is one sticky bacterium," Weiner says. He tested the idea and found that these bacteria produce polymers with substantial adhesive strength that could set in salt water, a discovery with clear commercial potential.

Breaking Down Complex Carbs

Weiner's scientific road soon took another turn. By the late 1980s, he had learned a great deal about how biofilms form, but realized that he knew little about how they are broken down. For that matter, he realized that little is known in general about how organic compounds in the marine environment degrade.

What happens, for example to giant kelp after it dies or to a massive algal bloom after its cells settle on the ocean floor? What about the shells of crabs, lobsters, zooplankton? Where do they go? These questions are central, he realized, to understanding how carbon flows through the marine environment. He began to steer his research program on this new course.

Complex carbohydrates (polysaccharides) do not readily break down into their carbon constituents. A significant amount of marine complex carbohydrates contain cellulose, but researchers had not specifically uncovered a marine cellulose degrader. We knew that carbon from the ocean was being released to the atmosphere, but didn't know how it was broken down, says Weiner.

When a huge die-off hit the Chesapeake Bay's cordgrass (*Spartina*) population in 1989, Weiner's colleague, George Andrykovitch from George Mason University in Fairfax, Virginia, enlisted his help to examine the microbial degradation process of the grass. Together, the two looked for an organism that might be responsible for *Spartina*'s breakdown. They recognized that one dominant microbe played a central role. Weiner called this bug, 2-40 — a name that refers simply to second batch, 40th colony.

As he studied this species of bacteria in greater detail, again with support from Maryland Sea Grant, Weiner and coworkers found that 2-40 produces a whole slew of enzymes specialized to break down not only the cell walls of plants but also chitin, the carbohydrate that makes up a crab's exoskeleton. The regulation of this enzyme production is complex, Weiner explains, but different steps in the process can be optimized to produce desired enzymes for commercial purposes. "The beauty of this is that it holds the record for degrading more complex carbohydrates than any bug ever detected," Weiner says.

With support from the Department of Energy, Weiner obtained the genome sequence of *Saccharaphagus degradans* 2-40. The bacteria proved a "veritable goldmine of genes," he says. While the genome independently verified that this bug produces a lot of different enzymes that degrade complex carbohydrates, it also revealed some surprises. Weiner's group found that the 2-40 genome also codes for antibiotics and a molecule that blocks the function of protein-digesting enzymes, which scientists call a global protease inhibitor.

The microbe 2-40 proved very difficult to isolate on its own, leading Weiner to suspect that it forms close relationships with other organisms. He knows that some of its closest relatives can only function in true symbiotic relationships. The quest now, he explains, is to discover what this organism is actually doing in nature.

But regardless of how 2-40 operates in the environment, its unique properties have clear commercial potential. Weiner and co-workers have already secured 6 patents from 2-40 that encompass a range of its functions. They've patented a slurry from the bacteria which can be used to degrade marine biofilms and have secured a patent for the protease inhibitor. They are currently looking to patent 2-40's ability to break down lignin, a large carbohydrate molecule that helps give plants their mechanical strength. With 2-40's genome sequence in hand, this science is really getting interesting now, he says.

Bird's Eye View

Over its long road, Weiner's career has run the gamut. He has zoomed in on very basic questions in cell biology, such as cellular aging. And he has panned all the way out to functional applications, like oyster aquaculture and marine adhesives. Now, as he brings his work on 2-40 to fruition, Weiner also has the unique opportunity to view his field from a big-picture vantage point. He currently works at the National Science Foundation

See Weiner, page 16

Weiner, *from page 15*

(NSF) as a Program Director within the division of Molecular and Cell Biosciences in the Biological Sciences Directorate. From this perspective he helps direct the flow of the peer review process, bringing him into contact with hundreds of grant proposals at the cutting edge of research in his field.

With the hindsight of a career's worth of experience in his discipline, Weiner finds that he now really enjoys the outlook on science that NSF provides. "You get a view of science that is different from what you've ever had before. You see it from different angles," he says. "You get to sometimes move it in a certain direction too."

While Weiner's enthusiasm for his life's work is unmistakable to those he encounters, the depth of it sometimes still comes as a surprise to him. "I found out something about myself when I got to NSF," he says. "I really love science."

In Memoriam

Kenneth Tenore, the director of the UMCES Chesapeake Biological Laboratory from 1984–2005, passed away on May 7, 2006. His absence will be felt not only by the CBL community, but also by the many individuals he touched in local, state, national, and international environmental circles. Among the legacies he leaves at CBL are improved facilities, an outstanding faculty, and highly regarded programs in environmental chemistry and toxicology. He was passionate about the ethics of science and about passing this knowledge on to students. He developed and taught a course in science and ethics with collaborators from the University of Notre Dame that was offered to graduate and undergraduate students at the University of Maryland and St. Mary's College of Maryland.


Harold Anderson

Dr. Tenore addressing REU undergraduate summer fellows in 2004.

During Tenore's directorship, he founded and directed the Alliance for Coastal Technologies (ACT), which is fostering the use of sensor technologies for environmental monitoring in coastal areas around the nation. He also led international marine research programs that involved scientists from the United States, Spain, and Portugal. At the time of his death, he was leading the Navigator Project, an international effort supported by the National Science Foundation and the Luso-American Foundation to characterize and compare the ecology of coastal seas around the world.

Friends and colleagues of Dr. Tenore have established a memorial fund in his name to support graduate education at CBL. Donations can be sent to: The Dr. Kenneth R. Tenore Fund for Graduate Education, CBL, P.O. Box 38, Solomons, MD 20688. Checks should be written to the University System of Maryland Foundation, Inc.

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