Scripps researchers set the stage for a bacterium's leading role in science

EXPLORATIONS FALL 2005

Every Microbe Tells a Story

DIPPING THEIR BEAKERS INTO an ocean full of stories waiting to be told, a team of researchers at Scripps Institution of Oceanography is chasing a suspenseful plot line.

Microbiologists Douglas Bartlett and Farooq Azam have as a protagonist a bacterium called *Vibrio cholerae*. It is a ubiquitous microbe that is normally benign, but has the capacity for havoc should it produce certain toxins like the one that causes cholera in humans.

For a setting, the researchers in Bartlett's lab have the coastal waters of San Diego, where they collect samples of *V. cholerae* on a regular basis. Just off the beach, the everyday lives of cholera bacteria and their cousins play out through millions of interactions with their neighboring microbes.

The scientists have a plot device that turns on the cholera bacteria, which suddenly become virulent by acquiring genes that code for lethal toxins.

How the story is resolved is anybody's guess.

"It's possible that if the strains of *V. cholerae* that we have out here just acquired a few more genes, then they could make us sick," Bartlett said. "They're not far removed perhaps from having the ability to infect us."

The story of marine microbes like these is of increasing interest to biologists—and not only when pathogens such as cholera are involved. Despite the fact that they exist at the microscopic level, microorganisms play a role in everything from regulating the planet's carbon cycle to maintaining the ocean's chemistry to providing life-saving drugs.

In this area, genomics has come to play a key role. Bartlett marvels at how a field barely in its first decade of existence is laying bare the exact genetic composition of marine microbes, allowing scientists to annotate the genes and classify them by function. Following a basic genetic blueprint, he and students like Alix Purdy are tracing how *V. cholerae* genes create proteins that carry out functions ranging from fending off predators to establishing colonies in the bodies of unsuspecting hosts. In the literary sense, think of Bartlett and crew as being in charge of character development.

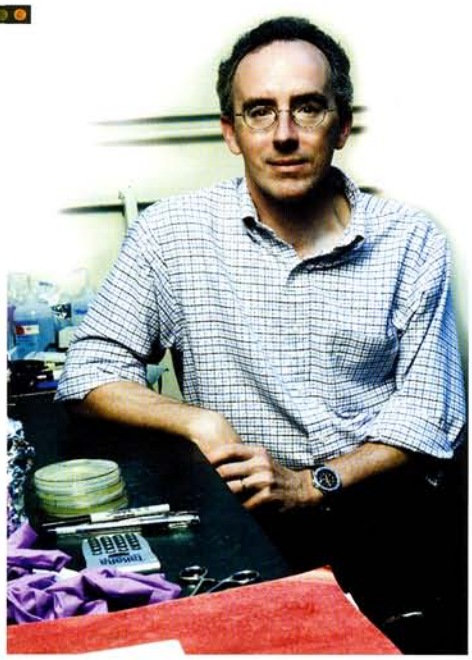
KEY TERM

COMPARATIVE GENOMICS: Field of study that compares genomes of different organisms to understand relationships between the organisms and to predict functions of newly discovered genes.

SPLIT PERSONALITY

Bartlett and those in his lab have rich source material to work with. Cholera bacteria have a notorious reputation forged over centuries. Even before German physician

Graduate students Simran Saini (left) and Alix Purdy collect cholera bacteria in brackish waters near Torrey Pines State Beach.



Douglas Bartlett

Robert Koch identified the strain in 1883, the bacteria had been the cause of the deaths of thousands of people in a string of outbreaks throughout the nineteenth century.

As recently as 1991, during an El Niño year that saw unusually warm ocean temperatures, a *V. cholerae* outbreak originated in Peru and spread north to Mexico, killing 11,000 people. Most of the spread was due to people drinking unclean water and eating infected food, which introduces the bacteria into the body in quantities sufficient to give it a foothold in the digestive tract.

Causing diarrhea and dehydration in humans is but a side pursuit of *V. cholerae*. Most individual microbes do not contain cholera toxin, the specific protein that makes only some virulent. So what makes the bacteria go wrong, acquiring and "turning on" the genes that code for production of the toxin?

The short answer is that scientists don't know yet. Despite the best efforts of Scripps researchers, they have not been able to infect a single marine organism with *V. cholerae* in numerous experiments. It means the toxin found in some bacteria has no known natural function. But using genomics and traditional lab chemistry, they are coming up with the means to answer such riddles.

Bartlett, Azam, and their students have made inroads into this mysterious microscopic world. The *V. cholerae* found around San Diego have neither the pili—structures that let virulent strains attach to tissue in digestive tracts—nor the specific cholera toxin protein. In a surprising find, Purdy discovered that the microbes often carry a toxin associated with a completely different genus of bacteria, one that frequently infects lung tissue in cystic fibrosis patients.

Before an epidemiologist can decipher the medical significance of this information, research is needed to understand the ecology of the habitats in which the cholera bacteria live. Researchers have known that *V. cholerae*'s milieu is a crowded one. The microbe is preyed upon by protists, and other competing strains of marine bacteria keep *V. cholerae*'s population in check. In this neighborhood, the name of the game is adaptation; microbes swap out pieces of DNA like auto parts from a junkyard. At a genetic level, they equip themselves to ride out famines and exploit feasts.

Finding out exactly how these processes take place is the specialty of Azam, practitioner of what Bartlett calls with a hint of awe in his voice "Azamian

Farooq Azam

KEY TERM

MEGABASE:
A unit of DNA length equal to one million nucleotides.

science." Azam studies the ecology of microscopic ocean communities; that is, how organisms coexist, compete, reproduce, repel, and kill, often by throwing a seawater sample into an altered environmental setting and painstakingly following how different microbes respond.

For several years, Bartlett has been part of an effort at Scripps to help southern California communities understand what beach-closing sewage leaks might portend for human health. For the story of *V. cholerae*, he is writing the plot, determining with the help of genomics what kinds of activities are possible.

"Knowing a genome does not tell us what organisms do in their environment, but it does put powerful constraints on what they could or could not do," Azam said.

In a joint project, Bartlett and Azam are attempting to observe *V. cholerae* genes during red tides, nutrient-generated blooms of phytoplankton in the ocean that typically send algal populations spiking. They employ microarrays, containing several thousand different gene sequences laid out in rows and affixed to glass slides. Researchers isolate messenger RNA (mRNA) from a control *V. cholerae* population and a test *V. cholerae* population experiencing red tide conditions in the laboratory. Genetic activity associated with the red tide conditions will be reflected in the test mRNA.

By tagging the organism's genetic material with fluorescent dyes and literally washing it over the microarray, the researchers can see which genes become active. They can then deduce how those genes are important in functions related to the environmental change.

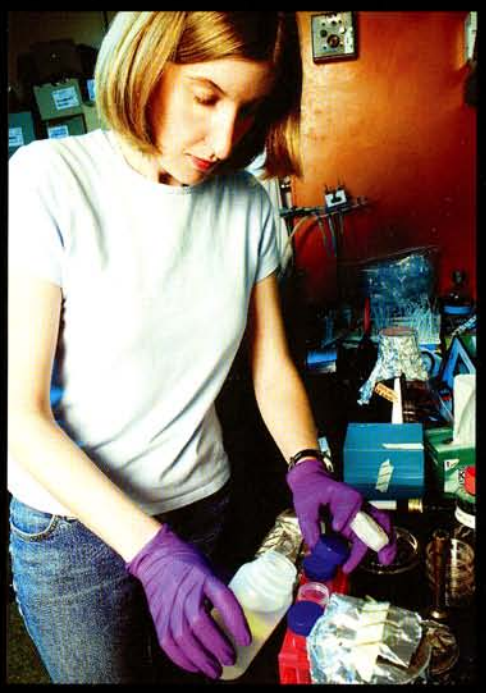
For example, with the new food supplied by the plankton in a red tide, the genes shown by the microarray to be active might be those that code for enzymes that help *V. cholerae* break down large molecules into digestible chunks.

Knowing the basics about how *V. cholerae* act in the presence of food or a predator or warmer or saltier water is a must for public health researchers. This information could someday warn them that environmental conditions are right for pathogenic bacteria populations to explode in a given area. It could signal that the microbes in a locality have been put in a position to take up toxin-bearing genes present in viruses and become carriers of disease.

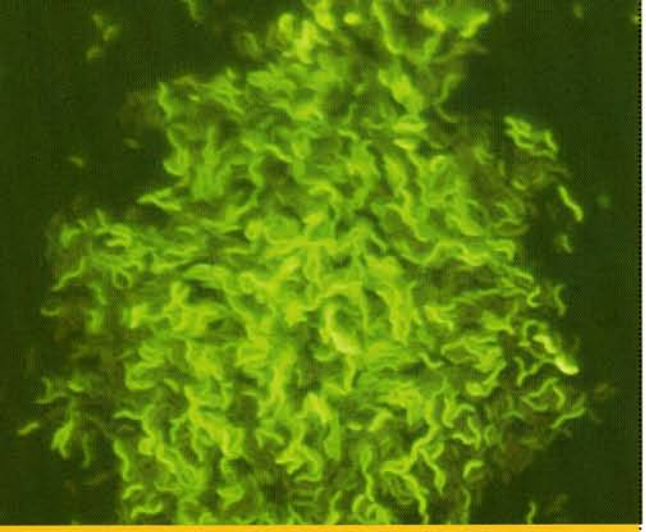
"New pathogens can evolve very quickly," said Purdy, a fifth-year graduate student. "People studying pathogens and virulence

KEY TERM

PROTEOMICS: The study of protein functions, including analysis of which proteins are expressed by an organism under certain conditions.



Left, *Saini and Purdy fill several collection bottles with seawater teeming with* V. cholerae *and other microbes.* **Right,** *In the lab, Purdy filters water samples prior to analysis or storage.*



Tagged V. cholerae

are starting to think more about the environment in which they live."

WRITING THE FINAL CHAPTER

One of those people is Joshua Fierer, chief of infectious diseases at VA San Diego Healthcare System. A collaborator with Bartlett, he may come up with what might be thought of as the moral of the story: how *V. cholerae*, *V. vulnificus*, and other members of the genus may afflict people in the future.

Fierer's own experiments found that the toxins within local cholera bacteria eviscerated lung tissue in mice. Like Bartlett and Purdy, he points out that this does not mean local cholera pose an immediate threat. Cholera is a hazard in places without clean drinking water or safe sewage systems. A swimmer in coastal waters around San Diego would have to drink seawater by the gallon to take in significant sufficient quantities of *V. cholerae* bacteria to be at risk. By then, they'd have other health problems more pressing than cholera.

Nonetheless, the adaptive nature of cholera could pose ever-changing risks to people who live in poverty-stricken stretches of Central America or Southeast Asia, where the disease is endemic. The final chapter of *V. cholerae*'s story has yet to be written.

"New pathogenic organisms can appear anywhere in the world and we have to be ready for them," Fierer said.

A TALE OF TWO *ATMOSPHERES*

ONE STRAIN OF *PHOTOBACTERIUM PROFUNDUM* lives in the water that laps at your feet when you stroll the beach, while another calls ocean depths of more than 2,500 meters (8,200 feet) home.

The difference between bacterial strains like these is analogous to that between dog breeds, but to do *P. profundum*'s extremes of adaptation justice, you'd have to compare one seriously large Great Dane to a Chihuahua's mini-me.

At Scripps, microbiologist Douglas Bartlett and fifth-year graduate student Federico Lauro are performing an exercise in comparative genomics on the two strains. Using the completed genome of the deep-sea microbe, known as *P. profundum* SS9, and gene sequences from its cousin 3TCK, they have found that the strains are 75 percent identical, but that the remaining 25 percent hosts traits specific to the habitat of each.

"Genomics is just giving us lots of global tools," Bartlett said. "We can ask lots of nitty-gritty questions."

Comparative genomics issues like this are more than just an interesting diversion for microbiologists, Lauro said. The genome of a deep-sea organism such as *P. profundum* SS9 allows researchers a greater understanding of the community of organisms that live at crushing pressures. The deep sea, regions of the ocean deeper than 1,000 meters (3,281 feet), is home to the largest portion of the ocean's microbial biomass despite being one of its least hospitable environments.

"Despite the fact that deep-ocean organisms dominate the biosphere, we know virtually nothing about them," Lauro said.

Getting to know these organisms better could have a variety of benefits. Such knowledge could increase the odds of finding hardy life-forms elsewhere in the solar system (or at least of knowing where to look). In addition, engineered deep-sea bacteria could tackle biotechnological tasks that require working in extreme pressure and cold.

That capability defines *P. profundum* SS9, a strain whose isolates at Scripps originated in samples collected in the Sulu Sea some 30 years ago by microbiology pioneer Aristides A. Yayanos. Although 3TCK has genes that allow it to perform functions like DNA repair in the presence of sunlight, SS9 has no genes or enzymes so activated by sunlight. (The name of the genus comes from the fact that some of its members are bioluminescent.)

The deep rewards compactness and economy of motion, as reflected in SS9's genes. In all organisms, long strings of proteins produced by cells must fold in specific ways to function. The proteins SS9 produces must fold inside the

Through filtering and separation in a centrifuge, graduate student Federico Lauro comes up with enough P. profundum *bacteria to be visible to the naked eye. The glob contains the collected genetic material of millions of microbial cells.*

clenched fist of the ocean's pressure. Thus, Bartlett and Lauro believe its genes code for proteins that fold with a minimal volume change.

In the lab, Lauro has shown that at reduced pressures typical of shallow waters, SS9 cannot carry out its genetic instructions. As a piezophilic bacterium, it grows and performs better at high pressure. For instance, SS9 cannot produce a functioning flagellum for swimming.

More answers will come in time. The research team still seeks "closure" on the 3TCK genome, which in the parlance of the field means filling in the gaps in information between large segments of gene sequences that have been analyzed in "shotgun" fashion and loosely assembled. Beyond that, researchers still have to deal with the special needs of extreme microbes such as SS9 that may have more in common with creatures from a Jovian moon than with their companion organisms on Earth.

"We cannot yet answer a lot of questions because of the way we have to ask them," Lauro said.