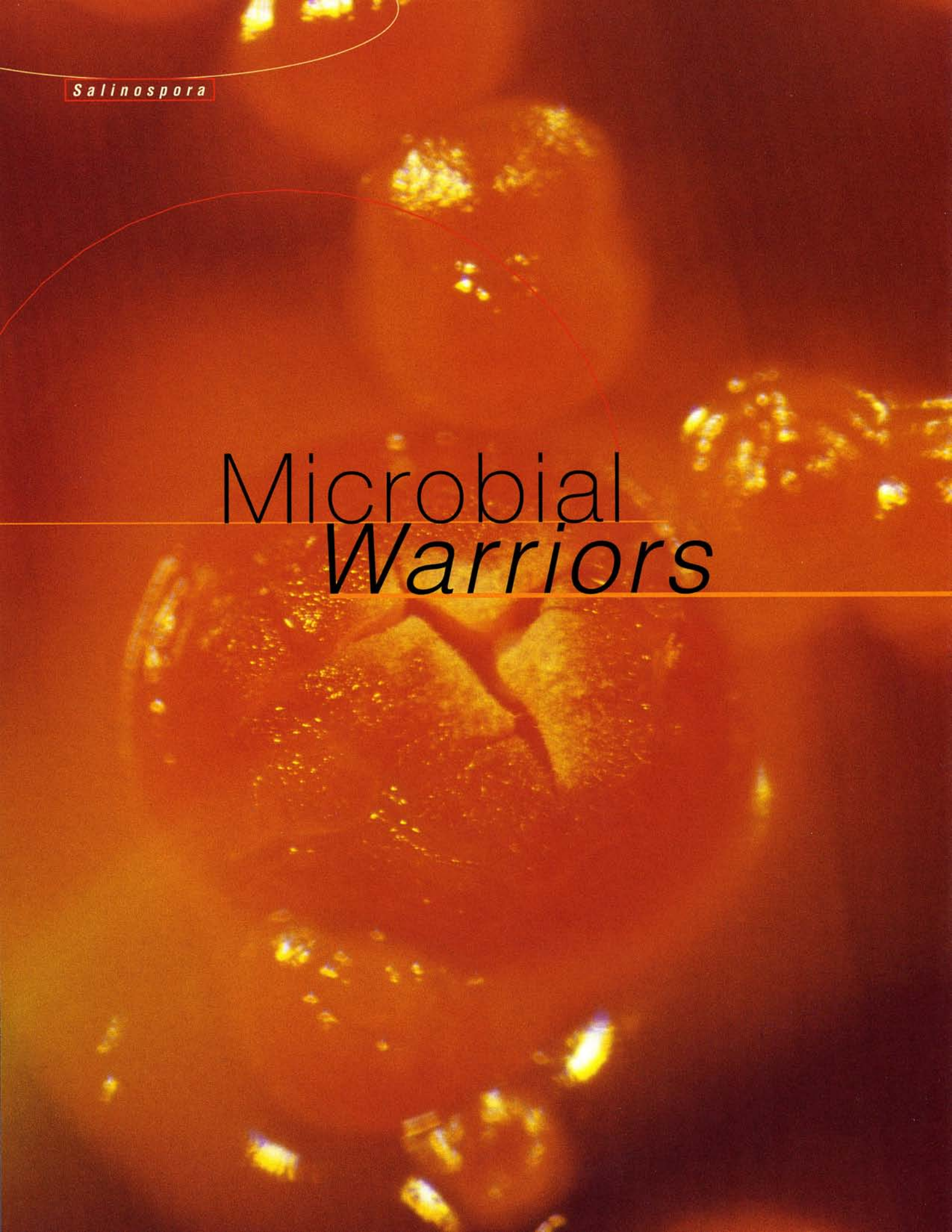


Salinospora

Microbial *Warriors*



BY ROBERT MONROE

THERE ARE STRAINS OF BACTERIA that can thrive under pressures that would turn a human into jelly.

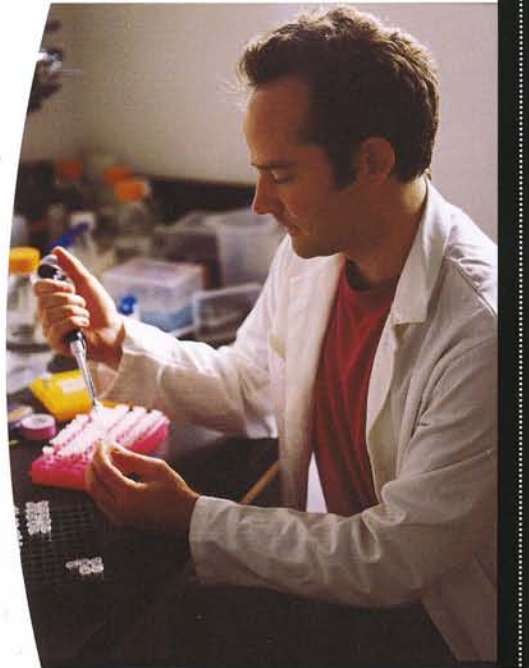
Other bacteria can live in pure acid. And some species have been found encased not only in blocks of ice but also in blocks of rock, while others live in boiling water.

So it might seem a little odd that scientists thought a bacterial order known as actinomycetes, the source of most of the world's natural antibiotics, couldn't handle a little salt water. Yet as recently as five years ago this belief was so strong that an announcement in 2002 by Scripps graduate student Tracy Mincer was as jarring as it was exciting.

Mincer found that a previously unknown genus of actinomycetes called *Salinospora* was indigenous to the ocean. The discovery is particularly timely because after decades of saving lives, land-dwelling strains of the bacteria are rapidly losing effectiveness as antibiotics. Since his initial discovery, Mincer has found at least 2,000 new *Salinospora* isolates, and experts in the field believe it could take a good 25 years for science to fully appreciate the magnitude of this breakthrough.

Across the Scripps campus, microbiologist Margo Haygood has won a battle nearly 10 years after being drafted into the war on cancer. For years, a mosslike marine invertebrate has been known to be the source of an anticancer drug called bryostatin. Haygood has determined that the compound more than likely comes from a specific microbe that lives in the larvae of the invertebrate. Regardless of the drug's final value, the techniques Haygood is developing for its production could be priceless.

Both researchers have opened fascinating new portals into the world of bacteria, a life form as invincible as it is commonplace. To microbiologists like Haygood, this research is prompting a new take on marine chemistry, challenging how scientists have looked at the ocean on a microscopic scale. To the rest



Left, Using a pipette to load samples, Tracy Mincer uses DNA sequencing to identify bacteria. **Opposite page,** *Salinospora*, the unlikely bacterium.



Above, *Salinospora* grows on a petri dish in the laboratory.



about other actinomycetes derived from 50 years of study may give researchers a head start.

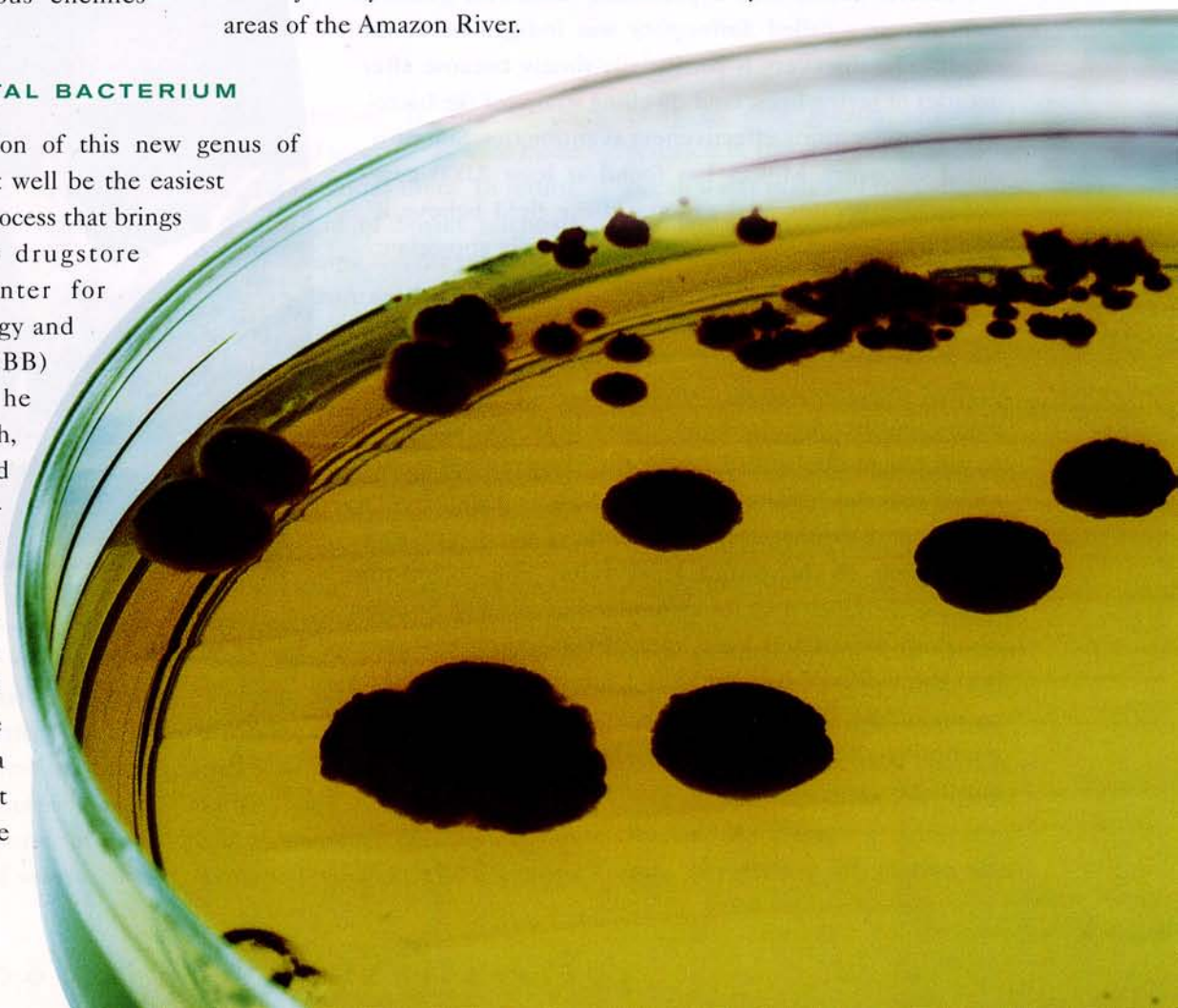
Actinomycete strains are so common and diverse that one isolate or another can be found in virtually any square inch of Earth's land surface. The 10,000 to 20,000 known isolates are found on leaves and bark, on rocks, and in dirt. They're so ubiquitous that for years drug companies encouraged employees taking trips to exotic lands to bring back soil samples. In the company laboratories, researchers sifted through these exported soils in search of strains different from their known cousins. With each new variation came a new chance to attack and kill pathogenic bacteria infecting people.

Antibiotic discovery has played out like a game of leapfrog for most of the past 70 years, because the near-infinite capability to adapt to their environs applies to both good and bad bacteria. Globetrotting drug makers, doctors, and patients take advantage of the ability of bacteria strains to kill within their own kingdom.

But in recent years, pathogens have gained the upper hand, learning to overcome medical treatment, as humans have misused and overused antibiotics. To keep up, researchers have gone to increasing lengths to look for actinomycetes, proven winners from which drugs with names ending in *mycin*, like vancomycin and streptomycin, have been derived. Their journeys have taken them to the Himalayas and the most remote areas of the Amazon River.

THE ACCIDENTAL BACTERIUM

Mincer's identification of this new genus of actinomycetes might well be the easiest part of an arduous process that brings any derivative to drugstore shelves. The Center for Marine Biotechnology and Biomedicine (CMBB) at Scripps, where he conducts his research, and San Diego-based Nereus Pharmaceuticals are partnering to develop medicinal uses for *Salinospora*. Testing drugs on people for safety and efficacy will take the better part of a decade, but the vast canon of knowledge

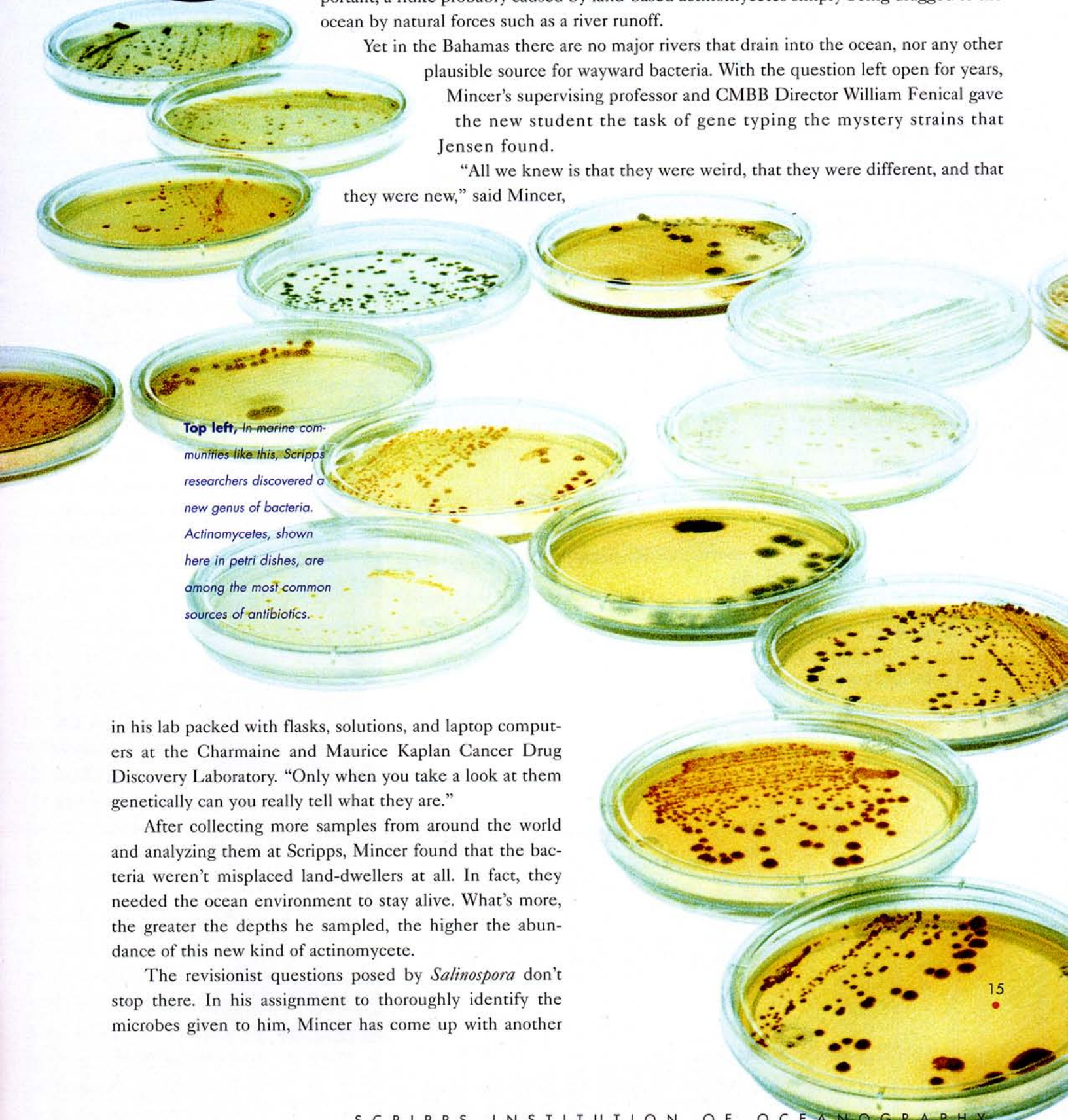




The story of *Salinospora* and Scripps began even before Mincer's arrival as a first-year graduate student in 1998. Nearly a decade earlier, a team led by CMBB's Paul Jensen had collected sedimentary samples in the southern Atlantic Ocean off the coast of the Bahamas and found actinomycetes embedded in the marine mud and coral rubble—a discovery previously thought of as impossible. Experts in the field shrugged off the find as unimportant, a fluke probably caused by land-based actinomycetes simply being dragged to the ocean by natural forces such as a river runoff.

Yet in the Bahamas there are no major rivers that drain into the ocean, nor any other plausible source for wayward bacteria. With the question left open for years, Mincer's supervising professor and CMBB Director William Fenical gave the new student the task of gene typing the mystery strains that Jensen found.

"All we knew is that they were weird, that they were different, and that they were new," said Mincer,



Top left, In marine communities like this, Scripps researchers discovered a new genus of bacteria. Actinomycetes, shown here in petri dishes, are among the most common sources of antibiotics.

in his lab packed with flasks, solutions, and laptop computers at the Charmaine and Maurice Kaplan Cancer Drug Discovery Laboratory. "Only when you take a look at them genetically can you really tell what they are."

After collecting more samples from around the world and analyzing them at Scripps, Mincer found that the bacteria weren't misplaced land-dwellers at all. In fact, they needed the ocean environment to stay alive. What's more, the greater the depths he sampled, the higher the abundance of this new kind of actinomycete.

The revisionist questions posed by *Salinospora* don't stop there. In his assignment to thoroughly identify the microbes given to him, Mincer has come up with another

hypothesis about their origins: Actinomycetes, the antibiotic source once thought to exist only on land, were probably native to the ocean to begin with. He examined base-pair splits, slight variations in genetic makeup that separate branches on an organism's family tree, and found a common ancestor to the ocean- and land-dwelling species dating back some 70 million years. This was also a period in Earth's history when the worldwide sea level had dropped some 300 meters (1,000 feet). Mincer's hunch is that the actinomycetes species left

Mincer found that *Salinospora* repelled fungus after the fungus landed on a petri dish filled with a pure culture of the bacteria.

"It was a very nice accident," he said.

How varied the *Salinospora* genus is isn't yet known. Mincer has collected different strains from various parts of the Pacific and Atlantic oceans, Caribbean and Red seas, and the Gulf of California. He has opened a door into a vast world. There could be other genera of actinomycetes or entirely new orders of bacteria with antibiotic properties of therapeutic value to humans just waiting to be found.

"It's like opening our eyes to the composition of the ocean," Fenical said. "To realize the magnitude of this discovery might take 25 years or more."

SUPPLY AND DEMAND

As with actinomycetes, development of bryostatin has been a work in progress for decades, and the finish line may be far away. In 1968, Arizona State University chemist Bob Pettit discovered that a chemical found in *Bugula neritina*, a mosslike marine creature in the phylum Bryozoa, could convert cancerous cells in the body back to normal by stifling their trademark rapid cell division. It did so while leaving normal cells alone, making it an exciting potential alternative to caustic and indiscriminate chemotherapy drugs.



Above, Former Scripps graduate student Seana Davidson collects phytoplankton, the staple of the *B. neritina* diet. **Far left,** Harvested on submerged panels, *B. neritina* provides the raw materials for bryostatin research. **Left,** The "Mud Snapper," an undersea soil sampler in action.

high and dry at that time had learned to adapt.

Preliminary testing has shown that *Salinospora*'s benefits might not be limited to antibiotics. There is evidence that it could shrink cancer tumors. (In April, the National Cancer Institute announced it had scheduled a cancer-fighting derivative, Salinosporamide A, for testing in animals.)

And, in a serendipitous instance reminiscent of Alexander Fleming's discovery of penicillin,



Bacteria researcher
Margo Haygood in her
laboratory at Scripps.



WHY DIRT

SMELLS LIKE **DIRT**

You will notice the scent when walking through a field turned muddy after a recent rainstorm. You might smell it in a handful of topsoil from your garden.

It's the particular odor we've come to consider the smell of dirt, a robust and, for lack of a better word, earthy aroma. But it's not coming from the dirt itself. Blue-green algae, also known as pond scum, and the bacterial order actinomycetes, which populate handfuls of dirt by the millions, are the true sources.

The bacteria and algae produce an oil compound known as geosmin (definitely no relation to jasmine). Scripps microbiologist Tracy Mincer says geosmin may be present wherever actinomycetes are found, but its function in nature is unclear.

Much scientific research regarding geosmin seeks ways to neutralize this familiar scent. It can impart musty flavors and smells to everything from tap water to bottom-feeding fish like catfish and earthbound vegetables like beets. 🌱

Researchers have gone on to identify 19 different bryostatin molecules since Pettit's discovery, but only one, known simply as Bryostatin-1, has been pursued as a potential cancer treatment.

Coming up with enough bryostatin to supply researchers has hampered the drug's progress from the beginning. Since Pettit's time, researchers have produced quantities of bryostatin by simply soaking large amounts of bryozoans in alcohol and extracting the chemical from what seeps out, a procedure they've had to rely on because until Haygood's

work, it was still unknown exactly where in *B. neritina* the bryostatin originated.

In retrospect, it's astounding that Pettit's team ever found enough of the chemical to be able to identify it in the first place. Consider that in the mid-1980s, the National Cancer Institute (NCI) needed a supply of bryostatin for testing, so commercial divers were contracted to harvest *B. neritina*. The divers scraped 13,000 kilograms (14 tons) of the ungainly invertebrates—which resemble small brown clumps of hair—from under-sea rocks in the Pacific Ocean off Long Beach, California. They gathered enough to fill a few swimming pools. From that mass, researchers extracted 18 grams of bryostatin, barely enough to fill a beaker.

"This is a product that had potential as a drug, and it was being held up for lack of supply," said Janice Thompson, the NCI official who commissioned the divers.

Thompson was also a classmate of Haygood's at Scripps. The two had discussed the supply problem off and on for many years before Haygood took it on as a research project in 1994. By that time, bryostatin's chemical

structure and its association with *B. nertina* larvae, which was known to bear special bacterial symbionts, were narrowing the list of potential sources. It became clear that the chemical probably didn't come from the weedy sea creature at all but from a microbial companion.



Technologies, a Carlsbad, California company devoted to finding marine-based pharmaceuticals and processes for producing them. The company needed a researcher with expertise in bacteria and the tools to identify them.

Haygood, a self-described “microbial chauvinist,” fit the bill. In her spartan office, with its Oriental rug and soothing water fountain, she reflects with admiration on the genetic ability of bacteria to deal with environmental conditions like acid, heat, and pressure.

Bacteria were the only living organisms on Earth for two billion years, enough time to develop a genetic diversity that allows strains to redefine their very essence in order to handle any kind of curveball the environment or scientists throw at them. Bacterial genes contain instructions written in enzymatic code that can be very simple, incredibly complex, and often rewritable to let the microbes deal with new environments.

The bacterium *Escherichia coli*, for example, is best known for the times when virulent strains have rendered undercooked fast-food burgers lethal. But *E. coli* is also genetically manipulated to keep diabetic individuals alive by producing insulin, a completely alien chemical, by adopting the insulin's genetic coding and reproducing it as though the chemical were its own. On a mammalian scale, it's like training a sheep to grow leaves.

“When the environment challenges bacteria, they just have so many more genetic resources,” Haygood said.

Haygood signed on, and CalBioMarine and the California Sea Grant College cosponsored her search for the bryostatin source.

“I just couldn't resist,” Haygood said. “It was a fascinating basic science puzzle.”

Nearly ten years later, Thompson still credits Haygood, then an untenured professor at Scripps, for taking on a research project that could have very easily flopped and hobbled her career.

“It was very brave. The probability of success with this problem was low,” Thompson said, “but she was pursuing academic lines of work that were important.”


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The possibility was important because it is generally easier to grow bacteria in large quantities than to grow marine animals. If a bacterial source could be found and exploited, then the supply problem would not be an issue.

Thompson left NCI in 1990 to help start up CalBioMarine

Top right, Sixteen tons of bryozoan and what do you get? About a small hand full of bryostatin. **Middle,** Margo Haygood. **Bottom,** Cotton candy-like larvae of *B. nertina* are home to an extraordinary bacterium.



FRIENDS IN NEED

In pill form, an actinomycete-derived antibiotic can stop infection caused by everything from cuts to eating spoiled food. In the wild, the bacteria help plants and other hosts ward off all kinds of invasions.

Scripps researchers Margo Haygood and Tracy Mincer, both investigating the therapeutic value of bacteria to people, are also interested in what these special bacteria bring to the symbiotic relationships they form in the wild.


For instance, the bacterium "*Candidatus Endobugula sertula*" doesn't seem to do any of the tasks normally associated with marine microbes. To its host, the larvae of a bryozoan called *Bugula neritina*, it does not provide food, a means of making food, a source of protective bioluminescence, or other common benefits.

What it must provide, researchers speculate, is a chemical defense, possibly what stops the larvae from being eaten by fish. While medical researchers are interested in the cancer drug bryostatin that comes from "*E. sertula*," Haygood believes that is the same chemical that the bacterium is using to protect its host and that the chemical's role in both settings is important to understand.

"Chemical diversity is what gives us a chance to find different kinds of beneficial drugs," Haygood said. "You have this evolutionary driving force to allow plants and animals to defend themselves, and bacteria are extremely good chemists."

Like "*E. sertula*," a newly discovered ocean-dwelling genus of bacteria called *Salinospora* probably also chemically protects its hosts—plants and algae—against fungi and other attackers just as its land-based cousins do. On land, hosts reward strains related to *Salinospora* with food in the form of slimy sugary polymers secreted at the plant surfaces where the bacteria live.

Salinospora's potential as a drug source is exciting, according to Mincer, but to the student of microbiology, its role in real life is interesting enough all by itself.

"I wanted a group of organisms that could be a resource for drug development," Mincer said. "I didn't want to chase down a bacterium that wouldn't have downstream applications. This is more than I had ever hoped for." 

THERAPY BY THE THIMBLE

Haygood found the source of bryostatin by making an inventory of all the bacteria associated with *B. neritina*, although she began her investigation with a prime suspect in mind. There was one particular symbiotic microbe in *B. neritina* larvae that had been discovered in 1981 but was never named. Like

Mincer's bacteria, the symbiont had a purpose that wasn't immediately clear—but its presence was. (See "Friends in Need" on p. 19.) Unlike their parents, which are covered by all kinds of bacteria, the larvae possessed just the one. Haygood was instantly intrigued by the association.

Her team compared amounts of

bryostatin in its samples to amounts of bacteria and tested which strains among the bacteria were capable of making bryostatin-like molecules. By 1997, with evidence of the larval bacteria as the source mounting, they received a name, "*Candidatus Endobugula sertula*." By 1999, Haygood's team felt it had enough evidence to make its case in writing. The resulting paper was published in October 2001.

The connection between "*E. sertula*" and bryostatin is of crucial importance to cancer researchers, who now know where to look for the drug.

Beyond that, however, the link could be an enormous revelation: It could be that many other ocean chemicals that have interested scientists for years come not from endangered marine animals and plants themselves but from the plentiful microbial symbionts that come with them. The most important chemicals in the sea might come from a source that is easier to exploit.

In choosing the bacteria's name, Haygood is following strict taxonomic rules. The word *sertula* is a Latinized reference to its wreathlike shape, and *Endobugula* acknowledges its host. But it is the first name, *Candidatus*, that is perhaps most important. It is an indication that the strain isn't an official species yet. In order for science to confer that status upon it, researchers need to be able to culture it.



Growing actinomycetes in the laboratory.

Right, Tracy Mincer with incoming Scripps graduate student Erin Gontang in the Kaplan Laboratory.



That brings Haygood and CalBioMarine to their current challenge: making more “*E. sertula*.” Though entire segments of science and industry cultivate bacteria for various purposes every day, the fragile “*E. sertula*” isn’t ready to become a workhorse yet. Haygood is attempting several methods of creating more of the microbe—or at least of the bryostatin it produces—and none of these processes is easy.

For instance, the team is trying to culture “*E. sertula*” in the lab but is finding that the bacteria die quickly when separated from their symbiont, *B. neritina*. Another route would be to simply harvest more *B. neritina* to accompany it. Although CalBioMarine has experimented with aquaculture, it is a prohibitively expensive prospect. Company president Dominick Mendola estimates that setting up and operating an aquaculture program for *B. neritina* would run about \$10 million.

These problems have made another route far more attractive: cloning the bryostatin gene from “*E. sertula*” into another bacterium. Cloning would allow another, more familiar bacterium to accept the bryostatin recipe and begin produc-

ing it. Haygood is pursuing the cloning technology that CalBioMarine has licensed.

“There may be a fundamental reason why you can’t grow the bacteria outside of the host,” Haygood said. “Unless we suddenly get really lucky with the cultivation, I’m betting that the cloning will work first.”

Currently Haygood is identifying and isolating the genes associated with the enzyme at the core of all bryostatin molecules. Once that enzyme, known as the bryopyran ring, can be produced by industrial bacteria, bryostatin research could expand considerably. There are 18 other forms of bryostatin, and any one of them could be more effective than Bryostatin-1. Haygood’s research could bring down the last barriers to affordable development of this family of drugs.

“Maybe some of them are even better than Bryostatin-1,” Haygood said. “The thing is, once you start with a particular molecule, you can’t switch midstream.”

Regardless of bryostatin’s fate, the research has already deepened scientists’ appreciation for things microbial, causing investigators to marvel as they take baby steps through bacteria’s vast miniature universe.

“Margo is tackling something that is very difficult,” said Cherry Herald, one of the co-discoverers of bryostatin, “and we all know that.” 