Medicines from Marine Microbes?

by Cherie Winner

To look for chemicals with pharmaceutical potential, a small pouch filled with resin beads is put into a flask with marine microbe cultures. The pouch works like a tea bag in reverse: Instead of sending flavorful molecules into the water, the beads soak up compounds made by microbes.

Microbes in the ocean could one day help doctors combat the deadly disease cystic fibrosis (CF), said Tracy Mincer, a microbiologist at Woods Hole Oceanographic Institution (WHOI).

Mincer studies the chemicals that marine microbes make to do such jobs as communicating with each other or warding off predatory protozoa. In October 2011, the Flatley Discovery Lab (FDL) of Charlestown, Mass., awarded Mincer a $1.2 million grant to provide thousands of chemicals for screening as potential drugs to treat CF.

The concept of using chemicals made by bacteria or fungi to treat diseases is hardly new. Penicillin came from the bread mold *Penicillium* and streptomycin came from bacteria known as actinomycetes, to name two well-known examples. But looking for medically valuable substances in the ocean *is* relatively new—and very promising. Each kind of marine bacteria produces possibly hundreds of small molecules called secondary metabolites, most of which have never been studied before.

"We're trying to provide the highest-quality microbial extracts from a diverse array of microbes that are producing small molecules that are unusual, different, and that might 'hit' the target," Mincer said.

The target in this case is a protein called cystic fibrosis transmembrane conductance regulator, or CFTR, which pumps chloride ions out of cells. In a person with CF, the protein doesn't pump enough chloride, and the cells produce mucus that is much thicker and stickier than it should be. That clogs up passages, making it harder for sufferers to breathe, for example. The gooey mucus also presents an appetizing environment for infectious bacteria. Repeated, difficult-to-treat infections scar the lungs and are the most common cause of patients' premature death.

The disease varies in severity depending on which form of CFTR an individual has. Scientists have identified more than 500 forms of CFTR, each with a different mutation. Mincer and Flatley will focus on the most common mutation, which affects about 70 percent of those with the disease, or 21,000 people in the United States.

Scientists at Flatley Discovery Lab have developed an assay to test whether a given compound improves the function of this defective form of CFTR. Mincer has started collecting chemicals from bacterial and phytoplankton cultures already on hand at WHOI. Later he will collect new samples from a variety of marine environments, including hydrothermal vents, the open ocean, and salt ponds.

Mincer said the ability of chemicals from bacteria to affect human cells isn't surprising, given their shared evolutionary history. A chemical that serves one function in a bacterium may not be needed by higher organisms to do the same function—but the higher organisms may retain the ability to make the chemical or respond to it.

"Nature does this a lot," said Mincer. "It's sort of a parts shop. Stuff gets re-purposed for some new use."

Mincer and technician Kristen Rathjen will purify compounds that give promising results in the FDL assays and determine their structure, which could provide clues to their function in nature. He also plans to study what the metabolites do for microbes in the environment. He's especially eager to find out whether the bacteria make them all the time, or only when stimulated by specific environmental conditions such as a change in temperature or the presence of predators.

"This is another aspect of the project I'm really excited about," he said, "Perturbing the bacteria by saying, 'Look, here's something that is going to eat you; what are you going to do now?'"

Bio-prospecting for potential drugs

This project is not Mincer's first foray into pharmaceutical discovery. During his graduate work at Scripps Institution of Oceanography, he studied marine actinomycetes, a group of bacteria that makes a variety of potent chemicals to ward off organisms that graze on them, such as protists. One day he noticed that a colony of actinomycetes growing in the lab was surrounded by dead protists. He realized that compounds secreted by the bacteria might have been responsible and passed a bacterial extract to lab

Growing microbes to harvest potential drugs

1. WHOI technician Kristen Rathjen grows cultures of marine microbes in a pale beige nutrient broth for a week. The broth takes on the colors of natural pigments of the microbes. The strain of microbe in the green fluid was photosynthetic.
2. Compounds produced by the microbes stick to tiny resin beads inside a "tea bag" pouch.
3. To remove the compounds from the beads, Rathjen places the pouches in an organic solvent to create crude extracts.
4. Each 1-liter culture yields 3 to 5 milliliters of crude extract containing from 300 to 2,000 unique compounds. The extracts retain the distinctive colors of the cultures they came from. The crude extracts are run through a High Performance Liquid Chromatography column to separate the compounds by their polarity, a measure of their affinity for fats versus water.

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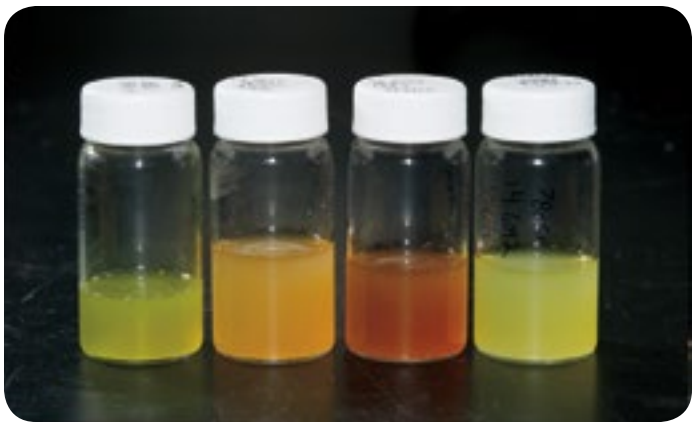

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All photos by Tom Kleindinst, WHOI

technicians who ran it through an anti-cancer screening test. It proved to contain a highly potent killer of cancer cells.

Mincer and colleagues isolated the compound responsible (by then called salinosporamide A), patented it, and determined its structure. It is now in phase 2 trials for treatment of multiple myeloma and a rare skin cancer. Along the way, a personal experience brought home the significance of the work to him.

"My in-laws in California have a dear friend who happened to be in the early clinical trials, and the drug has extended his life by several years," said Mincer. "To see that side of things really influenced me."

Despite such promising results, he said, it's been difficult for scientists to get funding from federal agencies to do the exploratory work that could lead to further drug discoveries. During his first year at WHOI, Mincer's requests for support from the National Science Foundation were firmly rejected. "Reviewers said it was a fishing expedition," he said. "It was hard; I really wanted to do this kind of work. What I really enjoy is being able to bridge that gap between drug discovery and basic research in ecology."

Support from the Flatley Discovery Lab will enable him to gather samples for his ecological work—perhaps enough of them to interest federal funding agencies—as well as provide ample material for the cystic fibrosis work. The grant extends over three years and will pay for the equipment and full-time technician to extract and fractionate the compounds. In return, Mincer and his team will send Flatley eight extracts per week—containing thousands of individual compounds—for testing.

That's a lot of potential drugs, which Mincer said is one of the biggest advantages of using microbes as miniature chemists: The more possibilities you start with, the better your chances of finding a winner. In recent years many large pharmaceutical companies have reduced or eliminated their natural products divisions, he said, choosing instead to invest in efforts to make potential drugs in the lab—but those attempts have struggled to generate enough good prospects to pay their way.

"It turns out, those methods don't even come *close* to mimicking what's possible from nature," said Mincer.