Microbes From 20,000 Feet Under the Sea

Japanese microbiologists are launching a major program to bring bugs from the bottom of the ocean back to land—and perhaps turn them into useful products

Tokyo—A COUPLE OF ACRES OF DISUSED dockland in the Japanese port city of Yokosuka, more than an hour's train journey from Tokyo, may seem an out of the way spot to launch Japan's next major thrust to lead the world in an area of high technology. But the technology is not exactly in the mainstream either. Rising above the industrial landscape is a new 6-storey research laboratory that will give Japan a unique facility to conduct a 15-year research project on deep-sea microbiology.

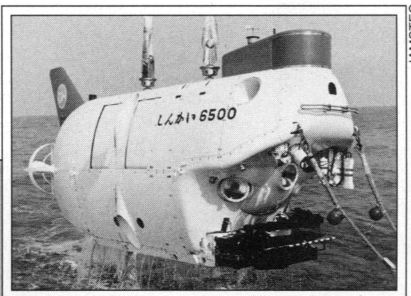
The new laboratory, provisionally named the Deep Sea Environment Research Institute, will be home base for the mother ship of Japan's Shinkai 6500—the world's deepest-diving manned research submarine. The plan is for Shinkai 6500 and other research submarines to bring back microorganisms, under pressure, from the bottom of the deep oceans. At the dockside, the organisms will be transferred to an automated microbiology laboratory where they can be cultured and isolated in water under hundreds of atmospheres pressure, temperatures from below freezing to above boiling, and pH ranging from acid to alkaline. With the aid of that apparatus, never before built on a large scale, Japanese microbiologists believe they will be able to gather information about the early evolution of life and deep-sea ecology—and at the same time search for genes with unique properties that might be usefully passed to the Japanese biotechnology industry. And if you have any doubts about the economic potential of research on bugs living in extreme environments, consider this: The key to polymerase chain reaction—the geneticists' new workhorse technology—is an enzyme cloned from a bacterium found in hot springs.

"People said I was crazy when I asked for 1000 atmospheres pressure in the laboratory," says Koki Horikoshi, senior researcher at the Institute for Physical and Chemical Research, and director of the fancifully named project DEEPSTAR (Deep-Sea Environment Exploration Program: Suboceanic Terrane Animalcule Retrieval). "The engineers said they have never done anything like it before."

For U.S. oceanographers, the scale of the project excites admiration—and envy. "It is much larger than the efforts that we have got going," said Craig E. Dorman, director of the Woods Hole Oceanographic Institution, while on a visit to Japan last month. "We have made similar proposals to NSF [the National Science Foundation] several times, but we have not been given the support," he added.

U.S. researchers are also envious of Japan's long-term commitment to the effort. There will be no yearly funding struggle for DEEPSTAR; $43 million over 8 years is already committed and Horikoshi is confident that a team of 30 scientists will continue the work for 15 years. In contrast, in the United States "the funding gets less and less," says Holger Jannasch, senior scientist at Woods Hole who pioneered many of the deep-sea techniques that the Japanese will use. "In a few years," he says, "the Japanese program will be ahead of us."

Ask Horikoshi how he pulled off such massive long-term funding and he tells a tale that makes sense only in Japan, where every government organization still has a feudal interest in guarding its own territory. The Japan Marine Science and Technology Center (JAMSTEC), to which the new institute will belong, has spent a fortune building a fleet of "the finest research submarines" in the world, explains Horikoshi. With the manned vessels Shinkai 6500 and Shinkai 2000, and the remotely operated Dolphin 3K (dive limits 6500, 2000, and 3000 meters respectively), only the very deepest troughs—3% of the ocean bottom—are beyond exploration. But the fleet sailed into some rough territorial waters. "JAMSTEC cannot study fish, because they belong to the Ministry of Fisheries," says Horikoshi, "and it can't study the structure of the sea or ports, since this is under the Ministry of Construction." So, he says, JAMSTEC decided to turn to microorganisms "because they belong to nobody."



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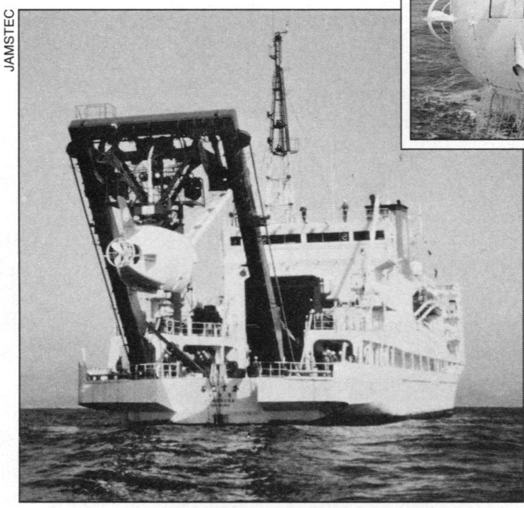

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To boldly go. *Shinkai 6500, the world's deepest-diving manned research submarine.*

Aside from the need to steer clear of other agencies' property, Horikoshi had good scientific and economic reasons for pitching a major project to study microbial life at the bottom of the sea. One potential goal is to collect new information on the origins of life. The basic logic, explains Bill Grant, a microbiologist from the University of Leicester who works on life in extreme alkaline environments, is that "the early surface of the Earth was an extreme environment so the organisms in present-day extreme environments might be their descendants."

So far, analysis of ribosomal RNA sequences from a variety of microorganisms that flourish around undersea hydrothermal vents, in waters close to the boiling point, place the most extreme "hyperthermophiles" at the very base of the evolutionary tree leading to the eubacteria, a form of life that branched very early—perhaps 3 billion years ago in the hot, pre-Cambrian environment—from the line that led to the eukaryotes. That suggests that the marine hyperthermophiles may be close to the ancestors of all life. With Shinkai 6500, Horikoshi hopes to explore vents at depths that other submarines—like the U.S. Alvin with its 4000-meter limit—have not been able to reach.

Horikoshi comes well prepared for the job having just completed a project dubbed

Superbugs in Waiting: Some Cautionary Tales

JAMSTEC

"Extremeophile" explorer. *Koki Horikoshi.*

Tokyo—Japan's plan to harvest microorganisms from the bottom of the sea may be the most ambitious of Koki Horikoshi's explorations of microbial life in extreme environments, but it is not the first. Last year, he completed the "Superbugs" project, a 5-year, $15-million study of microorganisms living in the world's most inhospitable environments. Brought back from his travels were scores of new microorganisms with the most bizarre properties, including bacteria that flourished in concentrated organic solvents, at pH 12, or at temperatures of 100°C. It may be decades—if ever—before these "extremeophiles" find a use. And if Horikoshi's experience is any guide, the applications might be surprising.

Take the case of the odd cellulase. Long ago, Horikoshi says, he set out to look for a bug that produced an enzyme that could hydrolyze cellulose in a high pH environment. Why? "It's hard to imagine now," he says, "but 20 years ago there were still few flush toilets in Japan and I thought the cellulase would be a good enzyme to treat human wastes. The undigested material is mainly cellulose." He found his bug in one and a half years, which was quick but nevertheless too late: The Japanese economic miracle had arrived and everyone was converting to flush toilets. "No one needed the enzymes," Horikoshi says. So he put them away in his refrigerator. But they were not completely forgotten. Ten years later one of Japan's biggest soap manufacturers asked to borrow them. The rest is marketing history: In 1988, the company launched "Attack" detergent with added ceullulase and now, says Horikoshi, the brand claims 60% of the Japanese laundry detergent market. The reason for its success? The cellulase from Horikoshi's bugs ignores the crystalline cellulose that makes up most of the cotton fiber but opens up amorphous cellulose regions where dirt can get trapped. Cottons wash whiter with the cellulase detergent—or so the advertising goes.

A similar strange twist of fate awaited another of Horikoshi's discoveries—an alkaline amylase. As amylases dissolve starch, Horikoshi thought it might be useful in automatic dishwashers to help remove rice (which is mostly starch) that is stuck on plates. But, alas, the Japanese public never took to dishwashers. All was not lost, however. A few years later, Horikoshi noticed that some of his amylases broke up starch without producing its constituent glucose molecules. Instead, the enzyme joined the glucose molecules together into a doughnut-shaped molecule called cyclodextrin. The center of the doughnut turns out to highly hydrophobic, meaning that it can trap a variety of compounds. A decade after his initial discovery, cyclodextrin is now being used in Japan to make "molecular capsules" that can wrap up fragrances or drugs, packaging them for slow release.

Magnetotactic bacteria isolated by Horikoshi's group may also find a new niche. In nature, they produce tiny particles of magnetite that help them to orient to magnetic fields. In the laboratory, the same enzyme systems can be used to produce a liquid magnet, a fine suspension of many tiny magnets in a solvent. Now scientists at Meiji Seika Kaisha Ltd. think they can link the magnetite particles to drugs to produce a targeted drug-delivery system—simply inject the drugs, place a magnet on a part of the body that needs them, and wait until they collect there.

These are the kinds of unexpected results that are driving Horikoshi's Superbugs project to the bottom of the ocean. And this time around, if he finds an enzyme that looks just right for washing dishes but ends up wrapping fragrances, he won't be surprised. ■ F.M.

"Superbugs," in which he searched for microorganisms in extreme environments on the earth's surface (see box). But going beneath the sea adds the complication of extreme pressure. At Woods Hole, Jannasch pioneered ingenious techniques for working on microorganisms that live in such an environment. He uses remote handling devices in a small chamber supplied with a high-pressure mixture of helium and oxygen to carry out the conventional steps for isolating microorganisms—dilution and plating onto Petri dishes containing agar gel. The Japanese are taking a different route, says JAMSTEC engineer Masanori Kyo, partly because they are aiming at a far larger scale and partly because they believe that deep-sea cultures are best grown in seawater. In their apparatus, Petri dishes and agar are replaced by a set of seawater cells in which the bacteria can be cultivated under high pressure.

JAMSTEC engineers have adapted lasers used to measure the amount of dust in the air to detect bacterial growth by the light they scatter. With a range of dilutions, it will be possible to pick out the cell with the greatest dilution in which there is still growth. With luck, that cell will contain just one kind of microorganism that can then be transferred under pressure to another part of the system to be grown up in quantity.

Until their new pressurized laboratory is completed in 1993, microbiologists now joining DEEPSTAR are using temporary laboratories near Tokyo. They have already begun dives with Shinkai 2000 in search of microorganisms that can survive gradual decompression and will still function at atmospheric pressure. One bacterium the researchers particularly wanted to get their hands on has already come their way. For years, they had noticed that there is little oil pollution in Japan's coastal waters, in spite of heavy shipping, and suspected that a bacterium capable of digesting oil was at work. A few weeks ago, a team of DEEPSTAR researchers announced that they had found such an organism at a depth of 1600 meters.

Like other bacteria capable of digesting oil, this one has obvious applications in tackling oil spills. But is also has an unsuspected property: It produces a surfactant that breaks up balls of oil into tiny digestible droplets. As surfactants reduce viscosity, Horikoshi hopes he might find an application for it in pumping oil out of wells. Says Horikoshi, "Though some very good surfactants have already been made using petroleum chemistry, they are sometimes toxic, causing environmental pollution. If we use these bugs it would be biodegradable."

For Horikoshi that first result is a sign that he launched his program at the right time. "There are still very few marine microbiologists," says Horikoshi, "perhaps 100 throughout the world. Now is the best time to start this field." And although he admits that he himself is a newcomer to deep-sea microbiology, he is confident about one thing. "Our research institute," he says, "will be the best." ■ FREDERICK SHAW MYERS AND ALUN ANDERSON

Frederick Shaw Myers is a free-lance science journalist based in Tokyo.