



Microbes, genomics and the Southern Ocean

Attempts to learn about who's out there and how they thrive in their frigid environment

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Winter wasn't quite over when the first science group arrived at Palmer Station for the spring season on Sept. 25. This microbiology-oriented project is led by myself, with the help of technicians Brandon Carter and Alison Kelley. Our mission is to capture literally billions of unassuming marine microbes and store them for gene expression analyses back at the Desert Research Institute in Reno, Nev.

Funded under the National Science Foundation's Life in Extreme Environments research program, our project is titled "Gene expression in extreme environments: extending microarray technology to understand life at its limits." No doubt with a title like that, a little explanation is needed.

Why study the microbes?

Marine microorganisms are the most abundant life form in the Southern Ocean. Their activities are essential to the ebb and flow of the Southern Ocean ecosystem. Let me explain...

The focus of our project is a diverse group of organisms inhabiting the plankton and sea ice that are known as the marine prokaryotes (cells without membrane-bound nuclei). These microorganisms fall into two distinct groups, the Bacteria and the Archaea.

In most of our minds, and as far as our eyes can tell even with the best of microscopes, these two groups of microbes would both be called bacteria. It turns out that the archaea are actually very different biochemically and genetically than the common "bacteria" that we associate with the term. Archaea are those life forms one might associate with thermal pools at Yellowstone, or deep-sea hydrothermal vents. They are often referred to as the extremophiles.

Archaea were only recognized to exist in oceans worldwide as recently as eight years ago, yet their sheer biomass (upwards of 40 percent of the cells in the deep ocean), indicates that they are likely quite important to carbon cycling in the marine ecosystem. The details of their exact roles remain to be discovered as of yet. Oceanographers, however, are quite familiar with the critical roles that bacteria play in the oceanic food web, many of which can only be performed by prokaryotes. For example, only prokaryotes can fix nitrogen, use inorganic chemicals as energy sources, transform reduced iron to a biologically available form and return degraded carbon otherwise falling to the seafloor back to the food chain.

Both bacteria and archaea are plentiful in Antarctic seawater, where you can generally find around 200,000 cells per milliliter (1 cubic centimeter or .03 fluid ounces). In sea ice, densities are more variable. Near Palmer Station over the past month we found densities ranging from 10,000 cells per milliliter of melted surface ice to 500,000 cells per milliliter in melted ice from the bottom layer. Defining the distribution of archaea in sea ice is part of our current work.

Environmental microbiology challenges

One of the most challenging, though essential, requirements for the study of life in extreme environments is investigating organisms in their natural habitat. While it may be simpler to study cultured microorganisms in a laboratory, it's not always



Photo courtesy of Alison Murray/Special to The Antarctic Sun

Alison Murray's microbiology team pulls sleds full of equipment and samples across the sea ice.

possible. At most, only 1 percent of these organisms have been successfully cultivated. By looking at the microorganisms in situ - that is, in their natural environment - we not only bypass the problem of cultivating them, we also get a far more accurate picture of what reactions and adaptations enable them to thrive in the environments they inhabit. It's these reactions, which can't literally be "seen" in such small organisms, that hold the key to understanding their survival techniques, and it's here that the genomic approach becomes useful. Molecular biology has revolutionized what we can learn about these microbes. We're utilizing technology that has largely been developed in biomedical research and learning to apply it to environmentally-oriented questions.

Genomics technologies to the rescue

Our current project applies technologies developed and utilized for the human genome project to address questions that remain poorly understood in the ocean. We're interested in developing the technology to detect gene expression in parallel for many different genes from Antarctic microbes collected directly from their environment. Genes that are actively expressed can be defined as those genes that are "turned-on," many of which are on all the time carrying out normal cellular functions.

A variety of other genes are expressed in response to changes in environmental conditions such as temperature, light, nutrient availability, or even other organisms. These are the genes we're interested in, and the ones that should help us develop a better picture of how Antarctic microbes adapt to their subzero environment. To identify which genes are expressed, we will construct DNA microarrays (i.e. genechips) which are specially designed microscope slides packed with anywhere from 500 to 10,000 different microbial genes robotically arranged in a grid pattern. With the help of an existing library of Antarctic microbial DNA prepared in an earlier field season by Ed DeLong, these microarrays will allow us to "match up" expressed genes from the Antarctic organisms we collected with known genes on the slides. This matching process will show which genes are being used to

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help the microbes survive in their frigid surroundings. The functional genomics aspect of this work is taking place in the molecular microbial ecology lab at the Desert Research Institute, while the sample collections and experimental manipulations are based out of Palmer Station.

With spring near, it's time to sample

You never know what the sea ice conditions will be during late winter-early spring in the Antarctic Peninsula region. This time around, though, we saw no sea ice on the southbound trip through the Bransfield and Gerlache straits; we were surprised to find Arthur Harbor and the surrounding region covered in ice. The inclement late winter weather of September and early October delivered consistently high winds, averaging 30 knots (35 mph) with gusts up to 74 knots (85 mph) one night, and inches upon inches of new snow. Periods of warm weather in September raised fears that the ice would not be suitable for travel, but the warm spell only lasted three to four days and temperatures dropped to the all-season low at Palmer the following week.

Once convinced the sea ice was safe enough to transport not only ourselves, but also our large seawater samples (200 liters, or 400 lbs.) and requisite gear, we set up two sampling stations in Arthur Harbor and one off of Bonaparte Point. We collected 21 ice cores and concentrated cells from more than 4,000 liters (1,040 gallons) of seawater. We also sampled the seawater and ice for descriptive data to characterize the habitat by taking subsamples for salinity, nutrients, chlorophyll and cellular density, then harvested the rest of



Photo courtesy of Allison Murray/Special to The Antarctic Sun

Alison Murray's field team and other scientists sample the ocean and its krill inhabitants at a sea ice station in Arthur Harbor. Pictured from left to right are Alison Kelly, Brandon Carter, Brett Pickering and Dan Martin.

the cells for microarray-gene expression studies. We were able to perform some molecular analyses at Palmer Station on some of the samples to determine the presence of Archaea, and to determine whether we could detect specific genes being expressed by microbes that are involved in the nitrogen and carbon cycles. In addition, we performed a variety of diurnal (full daylight vs. complete darkness) and stress-induced experiments varying the temperature or light levels that the marine microbes were exposed to.

Our hope is to develop a better understanding of the ecological strategies the Antarctic microbes utilize to thrive in their dynamic, subzero environment. Understanding the responses of microbial gene expression to changes in environmental conditions depends on identifying which tools (or genes) are being deployed from the vast reservoir stored in this tool box called the "environmental genome." With some luck, technological advances and productive field seasons - as this one has been - we may start unraveling the secrets that are stored in the tool box.

The long-term applications of this work may help identify new genes for biotechnological or pharmacological use, contribute to a better understanding of the requirements for life on this planet - or on other planets - and in the end help draw a more complete picture of how these plen-

tiful microbes are living and interacting with the inhospitable environments that they call home.

For references, and newsletters prepared on the Ice, please visit my Web site: <http://www.dri.edu/DEES/Faculty/Murray.html>, or contact me at alison@dri.edu, or Earth and Ecosystem Sciences, Desert Research Institute, 2215 Raggio Parkway, Reno, NV 89512 if you have any questions.