PROJECT SUMMARY

Gene expression in extreme environments: extending microarray technology to understand life at its limits.

One of the most challenging, though essential, requirements for the study of life in extreme environments is studying the organisms inhabiting these environments *in situ,* and understanding the unique aspects of biological life and adaptations required for survival in the variety of inhospitable environments that life has now been discovered to exist. The overall goal of the proposed research is to develop genomics approaches for studying microorganisms sampled directly from extreme environments. This approach circumvents the requirement for cultivation, and offers a powerful methodology for understanding facets of life in extreme environments that are poorly understood and difficult to study with conventional methods. This proposal is directed towards the area in the LExEn program which focuses on "Methods and Capabilities for LExEn Research". The objectives of the proposed work are to 1) sequence six large bacterial genomic DNA fragments isolated directly Antarctic marine psychrophiles totaling 240 kb of novel sequence representing phylogenetically divergent, and uncultivated microorganisms 2) construct two different types of DNA microarrays designed to identify genes being actively expressed in uncultivated microorganisms living in the sub-zero marine waters of the Antarctic, 3) optimize specific aspects of microarray technology for use with environmental samples, 4) develop a transferable methodology that will be useful for other LExEn researchers to access gene expression information directly from the natural environment. The Antarctic marine psychrophiles provide an excellent model group of extreme microorganisms for this work, since we know very little about their biological and functional diversity, or specific metabolic adaptations to life at –1.8 ^{o}C. Indeed, only six years ago the planktonic crenarchaeota were discovered to comprise a major fraction of the biomass in Antarctic surface waters in the early spring (DeLong et al. 1994). Subsequent studies have shown that this group has circumpolar distribution (Murray et al. 1999), is present throughout the water column (Massana et al. 1998), and has characteristic patterns of temporal variation (Murray et al. 1998). However, the planktonic crenarchaeota remain to be cultivated, and almost nothing is known of their metabolic capabilities. Fortunately, rapid progress in the genomics field has provided a variety of new technologies that dramatically increase the capabilities for high throughput analyses which will enhance the means for accessing information regarding uncultivated organisms. This study will make use of an Antarctic genomic DNA library comprised of large (40 kb) genomic fragments of planktonic archaeal and bacterial DNA created in an earlier project. The library contains sequences derived from a number of uncultivated organisms that encode psychrophilic gene products that contain vital clues for understanding specific psychrophilic adaptations of Antarctic bacterioplankton. The proposed research will utilize the Antarctic library to develop targeted and shotgun DNA microarrays. Targeted arrays will be comprised of genes that have been identified in sequencing efforts previously conducted (120 kb of psychrophilic crenarchaeotal sequence has been generated by Ed DeLong and colleagues), and in bacterial sequencing efforts described in this proposal. Shotgun arrays will be constructed with random fragments of selected 40 kb fosmids in the Antarctic library, making use of the high density capacity of microarrays. The application of DNA microarray technology to studies of life in extreme environments offers an outstanding opportunity for identifying new genes for biotechnological use, and for discovering specific adaptations to extreme environments by detecting genes that are uniquely expressed in the natural environment.