

Rapid Identification of Unexpected Bacteria Pathogens in Space Environments

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IN ADDITION TO BEING POTENTIAL HEALTH HAZARDS, BACTERIA can form biofilms that lead to corrosion or unacceptable odors. Bacteria can not be eliminated from the space craft environment and in fact are important components of biological waste treatment and hydroponics systems that will likely be included in long duration missions systems. Thus, it will be essential to have effective monitoring systems that report on the numbers and types of bacteria so that appropriate countermeasures can be instituted.

Particularly promising targets for use in microbial monitoring are organism specific sequences in the 16S *r*RNA sequence that has been experimentally determined in approximately sixteen thousand bacterial strains. The 16S *r*RNA is a naturally amplified target that can be detected by a large variety of methods. The project arose from the realization that this standard approach inherently requires that one anticipate what organisms might be present, which may not always be possible. Prior to the onset of the project, the principal investigator recognized that a better approach would simply be to determine the genetic affinity of organisms in a sample using a set of hybridization probes that are characteristic of various groupings. The purpose of the project was to determine if sufficient numbers of such signature sequences existed in the data set to implement the idea.

Results

The project was very successful. A useful signature quality function that takes into account both false positives and false negatives was developed and used to quantify the potential utility of all oligonucleotides of length N . This index allowed us to identify numerous oligonucleotides that are useful signatures for various taxonomic groupings. For example, In the case of 9-mers there were 19,715 sequences with signature quality over 0.6, 9,955 sequences over 0.8, and 6,908 sequences, which were perfect signatures for some bacterial grouping. Current work is focused on further understanding of the distribution of these signature sequences and the development of assays using oligonucleotide probes designed from the signature information.



Dr. George Fox—Tracing bacteria in space.

Consequences

There have been a number of direct consequences of this project. The specific results obtained were recently published in a leading peer review journal (Zhang, Z., R. C. Willson, and G. E. Fox. "Identification of Characteristic Oligonucleotides in the 16S Ribosomal RNA Sequence Dataset," *Bioinformatics* 18 (2002): 244-50). Data obtained was also instrumental in demonstrating the feasibility of our basic approach to microbial monitoring. As a result, we were able to increase our funding level from the National Space Biomedical Research Institute from \$48,000 per year to \$304,000 per year. The current award extends to November 30 of 2003 with a total budget of \$926,040. In addition, the new data obtained made it possible to submit a provisional patent application in 2001 that was recently upgraded to a formal patent application (Fox, G. E., R. C. Willson, and Z. Zhang. "Methods for Determining the Genetic Affinity of Microorganisms and Viruses," US and foreign patent applications filed January 26, 2002). Two students received master of science degrees as a consequence of our work on signature sequences. The first, Zhengdong Zhang (M.S., 2000 in Computer Science, "Identification of Characteristic Oligonucleotides in the 16S Ribosomal RNA Sequence Dataset") worked on the project during the funding period while the second, Usha K. Desai, (M.S., 2002 in Computer Science "An Object Oriented Interface for the 16S *r*RNA Oligonucleotide Signature Database") did important follow-up work by developing an effective interface to the signature data set. She also updated the signature database to include data from thousands of additional organisms.