Berkeley Lab Technology Could Help Areas Flooded by Katrina

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DNA ‘Phylochip’ Scans for Thousands of Disease-Causing Microbes

The flood waters that filled the streets of New Orleans in the aftermath of Hurricanes Katrina and Rita are now heavily populated with bacteria, viruses and other disease-causing microbes that could pose a grave risk to residents long after the waters have been cleared. An experimental technology developed by scientists with the U.S. Department of Energy’s Lawrence Berkeley National Laboratory (Berkeley Lab) holds promise for use in situations like this to provide a comprehensive picture of bacterial presence in the air, soil and water and enable authorities to track how that presence changes over time.

“Warm and stagnant bodies of water frequently give rise to large numbers of different bacterial pathogens and opportunistic organisms,” said Terry Hazen, a microbiologist who heads the Ecology Department of Berkeley Lab’s Earth Sciences Division and is a leading authority on pathogens in warm environments. “A key challenge is a comprehensive identification of all potentially deleterious organisms without prior knowledge of the diversity of pathogens that may be present.”

Originally developed for air sampling and still being tested by Berkeley Lab for the Department of Homeland Security, the Phylochip and its application to other media like those found on the Gulf Coast are pending further studies in soil and water.

Using a square-shaped DNA chip, the size of a quarter and packed with an array of 500,000 probes, Hazen and his colleagues can analyze a sample for the unique DNA signatures of 9,000 known species in the Phyla of bacteria and Archaea. The analysis can be completed within 24 hours from the start of the process.

“Our Phylochip makes its identification based on variations in the 16S rRNA gene, which is essential for protein synthesis and is present in all bacteria,” said Gary Andersen, a microbiologist who now heads the Molecular Microbial Ecology Group within the Lab’s Ecology Department. “Small regions of DNA base-pair sequence differences within the 16S rRNA gene can be used to distinguish different bacterial species, including pathogens. The advantage of this approach is that multiple pathogens can be identified simultaneously by targeting unique regions of the 16S rRNA gene sequence in the samples.”

The bacterial problems facing the coastal areas of Louisiana, Mississippi and Alabama, which were ravaged by Hurricane Katrina, and areas in Texas and Louisiana impacted by Hurricane Rita, are likely to persist for an extended period of time, Hazen said. As flooded areas dry out, some of the pathogens in the contaminated water will become airborne. Others will be absorbed or adsorbed into the soil. Some of these will percolate down into the groundwater, and others will remain near the soil surface where they could be desorbed through precipitation.

“Every time it rains, we could be seeing a spike in bacterial pathogen activity,” said Hazen. “The microbial population currently in that flood environment is complex and dynamic.”

With the Phylochip and special software developed at Berkeley Lab, health and environmental officials monitoring the areas flooded by Katrina and Rita could be provided with what Hazen characterizes as “snapshots” over a period of time. These snapshots would tell officials whether specific populations of bacterial organisms are increasing or decreasing. Samples collected from critical locations would have to be transported to Berkeley Lab for DNA extraction, polymerase chain reaction (PCR) amplification, and array hybridization on the Phylochip.

The key to the Phylochip's success is the 16S rRNA gene, which codes for a small substructure of the organelle in biological cells known as a ribosome. For bacteria, the DNA sequences that make up the 16s rRNA gene have been highly conserved throughout evolution, and can be used to positively identify individual species within the Phyla. These DNA sequences can also be used to infer natural relationships between different species.

In addition to bacterial pathogens, the 16s Phylochip would also reveal the presence in samples of bacterial species that are capable of degrading or transforming contaminants of concern. Under the right conditions, bioremediation and natural attenuation can be the safest and most cost-effective environmental remediation technique.

"We can validate the possibility of natural biodegradation and determine which species of biodegraders should be stimulated," said Hazen. "With this information, appropriate treatability studies can be designed for determining the best and most cost-effective remediation strategy."

The 16s Phylochip has been extensively tested under a variety of conditions and locales and has proven to be a more sensitive and accurate means of detecting bacterial species, including pathogens, than the current standard of using 16S rRNA gene clone library sequencing.

Hazen and the other scientists in the Ecology Department are experienced at tracking bacterial pathogens in tropical and subtropical waters, which pose a special challenge to health and environment officials. The standard indicators used to assess whether water has been contaminated with human feces, the primary source of pathogens that threaten human health, are the bacteria E. coli and Enterococcus. However, according to Hazen, these standard indicators can survive indefinitely in waters where the temperature approaches that of the human body, which means they do not necessarily reveal whether the human fecal contamination was recent, a critical point for assessing the risk of disease.

“Our research has demonstrated that other bacteria, such as Enterococci, Bifidobacteria and Clostridia, provide better indirect evidence of recent human fecal contamination,” Hazen said. “We also have experience with direct detection of pathogens like Vibrio vulnificus and Vibrio parahaemolyitcus, common in warm coastal water and already causing infections, some fatal, in Katrina-affected areas.”

Hazen and his colleagues have developed a number of techniques for detecting both alternative indicators and opportunistic pathogens. These techniques can be used to identify contamination sources and help health and environment officials better determine the real risk involved with contact and consumption of various water sources. In addition, they have shown how other technologies can also be brought to bear on the situation. For example, they have learned to use a micro-respirometer — a device designed to monitor real-time oxygen consumption, carbon dioxide or methane production — to determine within a few hours what would be the optimum conditions for achieving biodegradation of contaminants.

“A combination of the technologies we are developing could provide functional design criteria for the remediation of contaminated soil and water at a number of sites in the hurricane-affected areas,” Hazen said.

Hazen and the scientists in his department are now working on new version of the Phylochip that could be used to detect pathogenic viruses and fungi. They are also developing chips that can be used to test for specific pathogens, such as Yersinia pestis, the bacterium that causes the plague. This work was principally funded by the Biological and Environmental Research program of the Department of Energy's Office of Science.

Source: Berkeley Lab