Use of Oligonucleotide Hybridization Probes and Polymerase Chain Reaction to Determine the Source of Fecal Contamination in Karst Terranes

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ABSTRACT

Fecal contamination of surface and ground water remains a serious health problem in the U.S. and the world. This problem cannot be adequately addressed until the source of the contamination is known and remediated. At present, no standard monitoring technique exists that can identify the source of the bacteria. We have been working to modify and apply the molecular technique known as "oligonucleotide hybridization probes" to identify bacteria sources in the karst terrane of Middle Tennessee. The oligonucleotide hybridization probes can be used to target ribosomal RNA and DNA. Fecal bacteria unique to a host species can be identified using this molecular technique. Tests have been conducted using RNA hybridization probes that target universal sequences (all bacteria), *E. faecalis* (warm blooded animals), *Lachnospira multiparus* (ruminants), *Fibrobacter succinogenes* (ruminants), *Fibrobacter succinogenes* (ruminants), *Fibrobacter intestinales* (ruminants), *Bacteroides distasonis* (humans), *Bacteroides vulgates* (primarily human), *Bacteroides fragilis* (human) and *Salmonella* sp. (poultry & human pathogen). We were able to differentiate between these organisms in blind studies, but the concentration of bacteria had to be equal to or greater than 1,000 bacteria per liter before the cells were visually detected. The sensitivity was increased by using polymerase chain reaction (PCR) to amplify nucleic acid sequences on DNA (instead of RNA). PCR increased the sensitivity so that only 10 cells per liter were required for positive detection. Using PCR, we identified *Bacteroides fragilis,* a bacterium unique to humans, and *E. coli* in a water and biofilm sample collected from a karst spring-fed stream in Middle Tennessee. These preliminary results indicate this technique has potential to identify sources of fecal bacteria in hydrologically complicated karst terranes.

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