Evaluation of Methanobrevibacter smithii as a Human-Specific Marker of Fecal Pollution

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Microbial source tracking has historically focused on the origin of traditional enteric indicators including coliforms, enterococci, or Escherichia coli. Recently, questions of genetic variability and environmental persistence have encouraged researchers to search for additional animal specific indicators of fecal pollution. To establish a lower detection limit of 13 cells/ml. The Mnif method for M. smithii detection appears to be a rapid, insensitive, and reliable test for the presence or absence of human fecal pollution in recreational waters.

**Results**

**Materials and Methods**

**Primer Development & DNA Extraction:** Primer specificity was tested against 12 species of bacteria/ml was extracted using the MO BIO Ultraclean Soil DNA Extraction Kit (MO BIO Labs, Carlsbad California). In addition, a 100µl sample of methanogen culture was applied to a Whatman FTA® Classic DNA from human and animal feces was extracted from fecal samples using the UltraClean Soil DNA Extraction kit and the Powersoil DNA Kit (MO BIO) following the manufacturer instructions. Extracted DNA was amplified using primers designed from the nifH gene of M. smithii. Sensitivity assays showed that the Mnif PCR amplified the expected product in pure culture to 1pg, and sewer samples showed a detection limit in 5ng of total DNA. Sensitivity of the Mnif PCR assay for sewage-contaminated water was determined by adding a range of DNA concentrations from 1ng to 50ng in the PCR reaction. Detection limits for the Mnif PCR assay were determined by adding a range of DNA from 1ng to 50ng in the PCR reaction. Sensitivity of the Mnif PCR assay was determined by adding a range of DNA concentrations from 1ng to 50ng in the PCR reaction. Sensitivity of the Mnif PCR assay was determined by adding a range of DNA concentrations from 1ng to 50ng in the PCR reaction. Sensitivity of the Mnif PCR assay was determined by adding a range of DNA concentrations from 1ng to 50ng in the PCR reaction. Sensitivity of the Mnif PCR assay was determined by adding a range of DNA concentrations from 1ng to 50ng in the PCR reaction.

**Environmental DNA Extraction:** DNA from human and animal feces was extracted from fecal samples using the UltraClean Soil DNA Extraction kit and the Powersoil DNA Kit (MO BIO). Sensitivity assays showed that the Mnif PCR amplified the expected product in pure culture to 1pg, and sewer samples showed a detection limit in 5ng of total DNA. Sensitivity of the Mnif PCR assay for sewage-contaminated water was determined by adding a range of DNA concentrations from 1ng to 50ng in the PCR reaction. Detection limits for the Mnif PCR assay were determined by adding a range of DNA concentrations from 1ng to 50ng in the PCR reaction. Sensitivity of the Mnif PCR assay was determined by adding a range of DNA concentrations from 1ng to 50ng in the PCR reaction. Sensitivity of the Mnif PCR assay was determined by adding a range of DNA concentrations from 1ng to 50ng in the PCR reaction. 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