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Title: Source Identification of Fecal Pollution and Characterization of Microbial Communities in Urban Streams Using Molecular Techniques

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ABSTRACT

Fecal pollution and urbanization are the two major threats to the surface waters. Identifying the sources of fecal pollution is the top most priority in implementing remedial measures to mitigate fecal pollution. This study investigated the utility of using 16S-23S intergenic spacer region (ISR) of *E. coli* as a genetic marker to identify the sources of fecal pollution. *E. coli* colonies were isolated from five host sources and water samples from Accotink Creek. Length heterogeneity PCR (LH-PCR) and sequence analysis was performed to discriminate the *E. coli* isolates from various sources. This study also evaluated the effectiveness of urban best management practices (BMPs) in mitigating fecal pollution in urban streams. *E. coli* were selectively detected and enumerated using U.S. Environmental Protection Agency (EPA) validated membrane filtration method from the water samples collected from streams with and without BMP at the watershed level and along the stream channel. Additionally, this study examined the influence of urbanization and urban BMPs on physicochemical quality and microbial community of the receiving streams. LH-PCR and Multitag Pyrosequencing (MTPS) were used to characterize microbial community from urban streams with and without BMP, and forest streams. Physicochemical water quality parameters were collected *in situ* as well as analyzed in the laboratory. Sequence analysis of 16S-23S ISR distinguished *E. coli* isolates from various sources indicating its utility in using as a genetic marker in identifying sources of fecal pollution. Since 16S-23S ISR sequences were very closely related, LH-PCR analysis was not successful in discriminating *E. coli* from different sources. The presence of BMPs along the urban streams and/or in the watershed showed significant reduction in *E. coli* loading during the base flow and small storm events, although not necessarily below the primary contact recreation standard during big storm events. Poor water quality was observed in urban streams compared to forest streams, while urban streams with BMPs in the watershed had better water quality than streams without BMPs. Microbial community structure was different among urban streams with and without BMPs, and forest streams. MTPS corroborated the microbial community patterns discerned by LH-PCR fingerprints. A significant correlation was observed between microbial community structure and a combination of water quality parameters such as dissolved oxygen, soluble reactive phosphorus, total phosphorus, total suspended solids, nitrate, and temperature. This suggests that the microbial community structure is influenced by stream water quality.