

What Bacteria Could Tell: Physical Source Tracking Using Molecular Microbial Methods in Pine Creek Watershed, Allegheny County, PA

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As a tributary of the lower Allegheny River, Pine Creek drains 67 square miles diverse land use watershed in Western Pennsylvania. Microbiological stressors provided by diverse land uses, ongoing land conversion to residential use and flooding are significant problems in both Pine Creek main stem and its tributaries. Certain bacteria species may have some adverse effects on human health and cause waterborne diseases, including Cholera and Dysentery. Therefore, the potential human health risk posed by microorganisms is of concern in Pine Creek watershed. Now, known combined sewer overflows (CSOs) and failing septic systems are believed to be the main anthropogenic inputs of bacterial loads. Moreover, feces of native and non-domestic animals such as wild turkey and deer are also regarded as the source of bacteria contamination. The overarching goal of the present study is to characterize the Pine Creek bacteria community and identify the physical sources of microbiological contamination via multiply molecular microbiology methods.

A 16S rRNA clone library was constructed to screen bacteria community composition in the study watershed the percentage composition of 70 clones affiliated with different divisions and subdivisions was shown in Figure 1. Denaturing Gradient Gel Electrophoresis (DGGE) was applied to obtain the fingerprints of both bacterial 16S rRNA and 16S-23S Intergenic Spacer Regions (ISR). DGGE gel banding patterns were further analyzed with GelCompar II software

for the clustering and principal components analysis (PCA). Generally, the variations of water samples with similar bacterial fingerprints clustered together and could be well interpreted by the first two principal components. In addition, *Escherichia coli*. cell numbers were quantified by quantitative PCR (qPCR) methods and the results are compared with those obtained by using traditional plating methods.

In conclusion, high bacteria diversity was found in Pine Creek. *Bacteroides*, usually found in fecally contaminated water body, was identified from the clone library which indicated the existence of fecal contamination in Pine Creek. However, no clone was >97% close to indicator bacterium *Escherichia coli*., which suggested that *Escherichia coli*. was not the dominant species in the bacteria community. After carefully analyzing the water samples collected from Pine Creek and its tributaries every week, we found that weather conditions could significantly influence the bacterial loads. Interestingly, the DGGE banding patterns were very similar under dry weather conditions which revealed stable bacterial loads throughout the watershed. In contrast, under wet weather conditions, bacterial loads of upper and lower watershed, main stem and tributaries diverged from each other, which could be explained by the corresponding CSOs and SSOs nearby. Furthermore, the dominant loads that impact the downstream confluence could also be revealed by the fingerprint analysis. *Escherichia coli* qPCR results were comparable with the *Escherichia coli* plating results.

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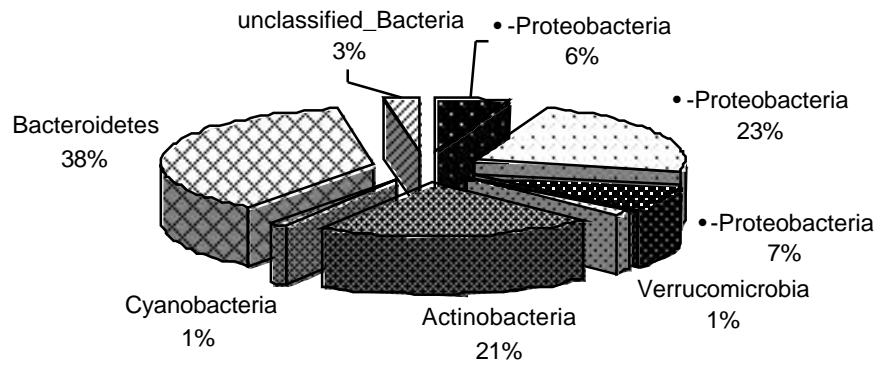


Figure 1. Percentage composition of clones affiliated with different divisions and subdivisions.