Identification of Heterotrophic Bacteria That Colonize Chloraminated Drinking Water Distribution Systems [Project #3088]

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OBJECTIVES:
The objectives of this project were to (1) identify the heterotrophic bacteria that colonize chloraminated distribution systems using bench-scale, pilot-scale, and full-scale environments, (2) determine whether bench-scale and pilot-scale chloraminated systems are adequate models of full-scale chloraminated distribution systems, in regards to heterotrophic bacteria, and (3) explore the development of an automated ribosomal intergenic spacer analysis (ARISA)-based screening test to identify culturable heterotrophic bacteria in drinking water distribution systems.

BACKGROUND:
Bacterial regrowth in chloraminated distribution systems encompasses the growth of ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB), and heterotrophs. While it has been demonstrated that *Nitrosomonas oligotropha* and *Nitrospira spp.* are ubiquitous AOB and NOB, respectively, in the drinking water environment, information regarding the identity of heterotrophic bacteria in chloraminated systems is limited. This project was aimed at filling this gap in knowledge by identifying heterotrophs using a 16S rRNA-based molecular biology approach.

HIGHLIGHTS:
*Actinobacteria*, *Bacteroidetes*, and *Proteobacteria* were the phyla representing the majority of 16S sequences in the library, with *Proteobacteria* being the most abundant.

There were statistically significant differences in the microbial communities of bench-, pilot-, and full-scale systems, indicating that water quality or system scale may introduce biases in simulating heterotrophic growth in distribution systems.

ARISA was not an unequivocal method to identify culturable bacteria in distribution systems, since one ARISA peak could be representing several microorganisms from different phylogenetic groups, and a single organism could be represented in an ARISA profile by more than one peak.

APPROACH:
A molecular biology approach based on the 16S rRNA as a phylogenetic marker was used to identify the heterotrophic bacteria. In addition, ARISA was used for community fingerprinting. Finally, ARISA was explored as a rapid screening and identification test for colonies grown in agar plates.

The following environments were studied:
Two bench-scale reactors simulating chloraminated and non-chloraminated environments, respectively.
Two pilot-scale distribution systems operated at the University of Wisconsin (UW) and two pilot-scale systems operated by the Louisville Water Company. The UW systems evaluated chloramine dose, while the
Louisville systems compared the absence of chloramines with chloramines plus chlorite.

Finished water and two water tanks in the distribution system at Louisville.

For all environments, only bulk water samples were analyzed.

RESULTS/FINDINGS:
Analysis of 16S rRNA gene sequences
A total of 1,371 partial 16S sequences and 197 full sequences were obtained. At the phylum level, *Actinobacteria*, *Bacteroidetes*, and *Proteobacteria* were identified in all environments. *Nitrospira* and *Firmicutes* were detected in five out of the nine environments. *Spirochaetes* were identified in two environments, while *Acidobacteria* were detected in only one environment.

Analysis of microbial community profiles
ARISA results from the two bench-scale reactors demonstrated that the microbial communities enriched in each reactor were not the same, in agreement with the operational mode of only one reactor receiving chloramines. The bacterial communities in the Louisville pilot plants were significantly different from the communities in the UW pilot plants, suggesting an influence of the water source or operational conditions. Full-scale samples revealed significant overlap of microbial communities in the two tanks sampled.

ARISA as a rapid screening tool for identification of culturable microorganisms
The predictions of ARISA peak identities were not unequivocal since it was determined that one peak could be representing several microorganisms from different phylogenetic groups, and that a single organism can be represented in an ARISA profile by more than one peak.

IMPACT:
The results of this study point to the presence of a “normal” distribution system microbiota, but also suggest that bench- and pilot-scale systems may induce biases compared to full-scale systems.

To explore the role of heterotrophs in the onset of nitrification, an understanding of whether nitrifiers are the first colonizers or whether chloramine-resistant heterotrophs initiate water deterioration is needed. For utilities interested in identifying heterotrophic bacteria growing in the distribution systems, cloning and sequencing the 16S rRNA gene is the most direct approach, while ARISA is a useful community fingerprinting tool for the characterization of a “normal” microbiota.

PARTICIPANTS:
Louisville Water Company and Milwaukee Water Works participated in this project.