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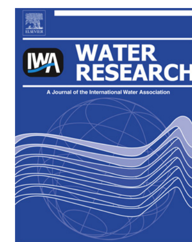

Molecular analysis of point-of-use municipal drinking water microbiology

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ABSTRACT

Little is known about the nature of the microbiology in tap waters delivered to consumers via public drinking water distribution systems (DWDSs). In order to establish a broader understanding of the microbial complexity of public drinking waters we sampled tap water from seventeen different cities between the headwaters of the Arkansas River and the mouth of the Mississippi River and determined the bacterial compositions by pyrosequencing small subunit rRNA genes. Nearly 98% of sequences observed among all systems fell into only 5 phyla: Proteobacteria (35%), Cyanobacteria (29%, including chloroplasts), Actinobacteria (24%, of which 85% were *Mycobacterium* spp.), Firmicutes (6%), and Bacteroidetes (3.4%). The genus *Mycobacterium* was the most abundant taxon in the dataset, detected in 56 of 63 samples (16 of 17 cities). Among the more rare phylotypes, considerable variation was observed between systems, and was sometimes associated with the type of source water, the type of disinfectant, or the concentration of the environmental pollutant nitrate. Abundant taxa (excepting Cyanobacteria and chloroplasts) were generally similar from system to system, however, regardless of source water type or local land use. The observed similarity among the abundant taxa between systems may be a consequence of the selective influence of chlorine-based disinfection and the common local environments of DWDS and premise plumbing pipes.

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1. Introduction

Federal US guidelines regulate the chemical and microbiological quality of treated drinking water as it leaves water treatment plants, but only highly specific monitoring, for fecal indicator bacteria, is required for water in the distribution system pipes (US 40 CFR Part 141: http://cfr.regstoday.com/40cfr141.aspx). Beyond the absence of indicators, little is known about the microbiology that is dispensed from point-of-use taps in water distribution systems, and there are no consistent regulatory standards for microbiological quality once the water passes the meter at a private residence or business establishment. Biofilms are known to be ubiquitous within DWDS and premise plumbing (Deines et al., 2010; Simoes et al., 2006; Yu et al., 2010), and can influence the

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planktonic assemblage of organisms delivered at the tap (Feazel et al., 2009; Szewzyk et al., 2000; Wingender and Flemming, 2011). Bacterial regrowth during distribution is dependent on dissolved and assimilable organic carbon concentrations, and can contribute to increased microbial complexity at the tap compared to finished water leaving the treatment plant (Liu et al., 2002; Niquette et al., 2001). Microbial dynamics from source to tap have been studied in a handful of isolated DWDSs (Eichler et al., 2006; Kormas et al., 2010; Vaz-Moreira et al., 2013), but there remains relatively little perspective on the specific makeup of the broader microbial complexity delivered at the tap with our public drinking waters, or how that complexity varies between different systems and different geographical areas.

Bacterial concentrations in DWDS waters are typically 10^6–10^8 cells/L (Hammes et al., 2008; Hoefel et al., 2003; Lautenschlager et al., 2010), but identification of those bacteria historically has depended on culture studies. Culture-based techniques are effective for determining the presence or absence of certain bacteria and have been used to study the effects of various physical and chemical parameters of water in DWDSs on cultivable bacterial loads and dynamics (Kalmbach et al., 1997; Srinivasan et al., 2008; Zhang and DiGiano, 2002). Since most environmental bacteria are not cultured without specific efforts (Hugenholtz et al., 1998), however, a culture-independent molecular approach is necessary for a broader assessment of the microbiology of these systems. Culture-independent sequence analysis of small-subunit (SSU, 16S) rRNA genes has proven useful for the identification of microorganisms present in DWDS waters and biofilms throughout the treatment and distribution process by both small scale Sanger sequencing of clone libraries (Eichler et al., 2006; Kormas et al., 2010; Poitelon et al., 2010; Regan et al., 2003) as well as multiplexed high-volume sequencing (Gomez-Alvarez et al., 2012; Hong et al., 2010; Hwang et al., 2012; Lautenschlager et al., 2013; Liu et al., 2012a; Zhang et al., 2012). These studies, however, have primarily focused on single distribution systems or storage facilities, simulated distribution systems in laboratory settings, or on biofilms within the DWDS and premise plumbing pipes and fixtures (Feazel et al., 2009; Hong et al., 2010; Liu et al., 2012a,b). Moreover, results from different studies vary considerably in the compositions detected and there are few generalities as to the microbiology consumed with public drinking waters.

In order to gain some perspective on the generalities and variations in the microbiology of point-of-use municipal waters, SSU rRNA gene sequences were used to identify the types of bacteria encountered in tap waters in seventeen different cities along the Arkansas and lower Mississippi Rivers. This is the first sequence-based microbiological survey of water delivered to the public across a large geographical area with different land uses, source water types and different disinfectants (chlorine vs. monochloramine). We hypothesized that these differences might influence the microbial makeup of the DWDSs, but that the nature of the distribution system, which is dark, low-nutrient, buffered in temperature and subject to the selective pressure of chlorine-based disinfectants, would be the primary influence on DWDS microbiology.

2. Materials and methods

2.1. Sample collection

Drinking water samples were collected from extensively flushed public taps in seventeen different municipalities along the Arkansas River from its headwaters in central Colorado to its confluence with the Mississippi River in southeastern Arkansas, and along the lower Mississippi River to its mouth in southeastern Louisiana (Fig. 1) during March of 2011. Cities sampled were chosen at random, approximately evenly spaced along these river drainages and were located in various land use areas, including forestlands, grazing areas, corn and wheat farms, rice farms, and wetlands. The cities are referred to with the abbreviation of the state followed by the number of the city in that state, e.g. CO1 – the first city sampled in Colorado; AR3 – the third city sampled in Arkansas, etc. Two samples were collected from each municipality on the outbound trip with varying degrees of geographical separation, usually <1 km, and those two locations were re-sampled on the return trip between two and sixteen days later. Sample sites were chosen at random, and included public buildings such as libraries, restaurants, medical centers, visitor's centers, or gas stations. In order to ensure that fresh city water from the water mains was sampled instead of water that had stagnated to any degree in the sampling premise pipes, each tap was flushed for several minutes until the chlorine residual had reached a maximum and stabilized according to SenSafe Free Chlorine Water Check test strips (Industrial Test Systems, Inc., Rock Hill, SC), or, in the case of chloraminated systems, for several minutes after the temperature had stabilized. For DNA analysis, 1.5 L of water was collected in sterile Nalgene high-density polyethylene (HDPE) bottles and immediately filtered through a 0.2 μm polycarbonate filter using a sterile filter funnel and vacuum flask setup. Filters were stored in sterile 2 mL microfuge tubes in liquid nitrogen for transfer to the laboratory. Temperature, salinity, dissolved oxygen, total dissolved solids (TDS), and pH were measured at the sampling site with a Hanna HI-9828 multiparameter meter (Hanna Instruments, Smithfield, RI). For cell counts, 45 mL of each sample was added to 5 mL 37% formaldehyde and stored at 4° C until counting. Formaldehyde-fixed cells were filtered onto 25 mm black 0.2 μm polycarbonate filters, stained with propidium iodide, and counted by direct fluorescence microscopy. A minimum of ten fields, evenly dispersed across the slide, were counted for each sample. Free and total chlorine concentrations were measured for each sample on the Hanna HI 96711 Free and Total Chlorine Photometer using HI 93701-0 free chlorine reagent and HI 93711-0 total chlorine reagent according to the manufacturer's instructions (Hanna Instruments, Smithfield, RI).

2.2. DNA extraction and amplicon generation

For purification of bulk genomic DNA from water samples, polycarbonate filters were removed from frozen storage tubes and placed into 2 mL extraction tubes containing 500 μL of phenol/chloroform (1:1 ratio), 500 μL lysis buffer (75 mM NaCl, 75 mM TRIS pH 8.0, 7.5 mM EDTA, 2.85% SDS), and ~0.5 g of

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