Appl Biol Chem (2017) 60(6):667–672
DOI 10.1007/s13765-017-0325-5

Online ISSN 2468-0842
Print ISSN 2468-0834

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Investigation of microbial communities in water dispensers

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Received: 10 September 2017 / Accepted: 24 October 2017 / Published online: 4 November 2017

Abstract Water dispensers remove disinfectant residues from tap water and thus are commonly present in Korean households; however, microbial contamination in water dispensers has recently become a major issue. To understand the occurrence of microbial contamination in water dispensers, we investigated microbial contamination in different dispenser types through heterotrophic plate count (HPC) and MiSeq-based microbial community analyses. Two newly purchased water dispensers were placed in a basement room and left for approximately 2 months; the HPC analysis indicated microbial contamination in the drinking water collected from these dispensers (160,000 and 48,000 CFU/mL, respectively). Taxonomic classification indicated that the basement dispensers were likely contaminated by freshwater bacteria, such as *Acidovorax* and *Methylotenera.* However, two dispensers located at the half landing and the first floor showed lower microbial contamination (110 and 78 CFU/mL, respectively). Furthermore, frequently used dispenser on the first floor showed higher HPCs on the faucet surface, which were classified as general oral bacteria like *Hyphobacterium.* We also observed that a deserted dispenser (6-year-old with no maintenance) placed on the half landing showed the least HPCs, although it seemed to have lost its filtration ability. Our results suggested that removal of disinfectant residues leads to an increase in the freshwater bacterial population in water tanks within dispensers, which could be avoided by frequent water circulation.

Keywords Disinfectant residue · Heterotrophic plate count · Microbial community analysis · Water dispenser

Introduction

Due to the fast industrial development, more than 95% of mines in Korea are abandoned [1]. Besides, heavy metals, organic materials such as herbicides [2] and livestock fecal materials [3] are known pollutants for drinking water and usually disinfected at wastewater treatment plants by chlorination and ozone oxidation [4]. However, residual disinfection by-products such as trihalomethane have been reported carcinogenic [5]. In Korea, water dispensers are commonly present in households and public facilities such as schools and hospitals, with the purpose of removing disinfectant residues from tap water. Most of the dispensers in Korea are of the direct-piping type, which filter tap water through filters (sediment and activated carbon) and membranes: Sediment filters remove relatively bigger particles, including sand and dust and carbon filters remove odor and smaller particles by chemical absorption, while membranes, employed in ultrafiltration (UF) and reverse

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13765-017-0325-5) contains supplementary material, which is available to authorized users.

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osmosis (RO), remove fine particles including microbes. Filtered tap water is then stored in a container within the dispenser, being heated or cooled before use.

Water dispensers can be contaminated with microbes and biofilms if not cleaned for a long duration [6]. In Taiwan, water dispensers in a hospital were found to be contaminated with *Legionella*, which lead to nosocomial neonatal Legionellosis [7]. Moreover, although air infection is usually suspected to be involved in the spread of the airborne pathogenic bacterium *Mycobacterium chimera*, causing nosocomial cardiovascular infection, Haller et al. [8] reported that *M. chimera* was prevalent in temperature-controlled water dispensers.

Since not all the drinking water present in dispensers is contaminated with bacteria, the mechanism underlying entrance and proliferation of microbes in water dispensers needs to be clarified. Hence, we investigated the microbial quality of tap water, water inside the dispensers, drinking water obtained from dispensers, surface area of the faucet, and air near the dispensers.

Materials and methods

Description of the dispensers

Four water dispensers were used in this study (A, B, C, and D), equipped with different types of filtration systems. Table S1 summarizes the dispensers used in this study with information including filtration systems, locations, and purchase date. Basement dispensers A and B were purchased in December 2016, only for this study, and thus they had never been used for drinking water, whereas dispenser C was purchased in 2010 and placed on the stairway between the basement and the first floor. This old dispenser's faucet was decolorated to blue, and there was no biofilm on the surface. Maintenance for dispenser C had never been carried out, and dispenser C had been hardly used for drinking water. Lastly, dispenser D was purchased in January 2016 and placed on the first floor and was frequently used for drinking water. All water samples were collected in January 2017.

Heterotrophic plate counts

Heterotrophic plate counts (HPCs) were obtained by spreading serially diluted water samples on plate count agar and incubated for 48 h at 35 °C. Water samples were obtained from tap water, before and after each filtration, and water storage tank within the dispensers. Swab samples were collected from the surface of faucet, then suspended in sterile water and used for HPC. Air bacteria HPCs were measured by filtering 100 L of air near each dispenser.

MiSeq-based microbial community analysis

Approximately, 200 mL water was filtered through 0.45-μm sterile membrane (Advantec, Tokyo, Japan) and used to extract DNA with PowerWater DNA isolation kit (MOBIO, USA). The DNA samples were sent to Macrogen Inc. (Seoul, Republic of Korea) for sequencing V3–4 region of 16S rRNA genes using Illumina's MiSeq platform (300 bp × 2) according to the manufacturer's instructions.

Obtained MiSeq reads were processed using MOTHUR [9] according to MiSeq SOP (https://www.mothur.org/wiki/MiSeq_SOP). Reads were aligned with Silva database [10], and UCHIME [11] was used to remove chimeric sequences. Taxonomic classification was done using the GreenGene database [12]. Operational taxonomic units (OTUs) were calculated at a distance of 0.03 using MOTHUR opticlust method. Cluster analysis was done based on Yue and Clayton coefficients with MOTHUR tree.shared subroutine. The differential abundance test was conducted using linear discriminant analysis effect size (LEfSe) [13].

Results and discussion

Quantification and taxonomic composition of bacteria in the dispensers

HPC analysis results (Table S2) and bacterial taxonomic composition are summarized in Fig. 1. *Pseudomonas* and an unclassified genus belonging to the family Rhizobiaceae were commonly found in almost all water and air samples obtained in this study. HPC analysis detected no bacteria in tap water from all the dispensers tested in this study. Dispenser A and B showed greater HPCs in water obtained from their storage tank and in the obtained drinking water. The relative abundance of the genera *Acidovorax,* which has been found in tap water and dispensers [14] and is capable of forming a biofilm [15], and *Methylotenera*, together known as freshwater bacteria [16], increased in dispenser A and B, respectively, suggesting that these bacteria proliferated in stored water since the dispensers were not in use and the water had not been circulated for a long time. Dispenser C showed the lowest HPCs among all dispensers, although it had not been in use for more than 6 years nor had it undergone any maintenance. It is likely that dispenser C lost its filtration ability, and perhaps, it failed to remove residual chlorine; consequently, bacterial growth may have been inhibited less as previously suggested [17]. Dispenser D, which was used frequently, also showed low HPCs compared to dispensers A and B. Relatively higher HPCs were observed in the pre-carbon filter

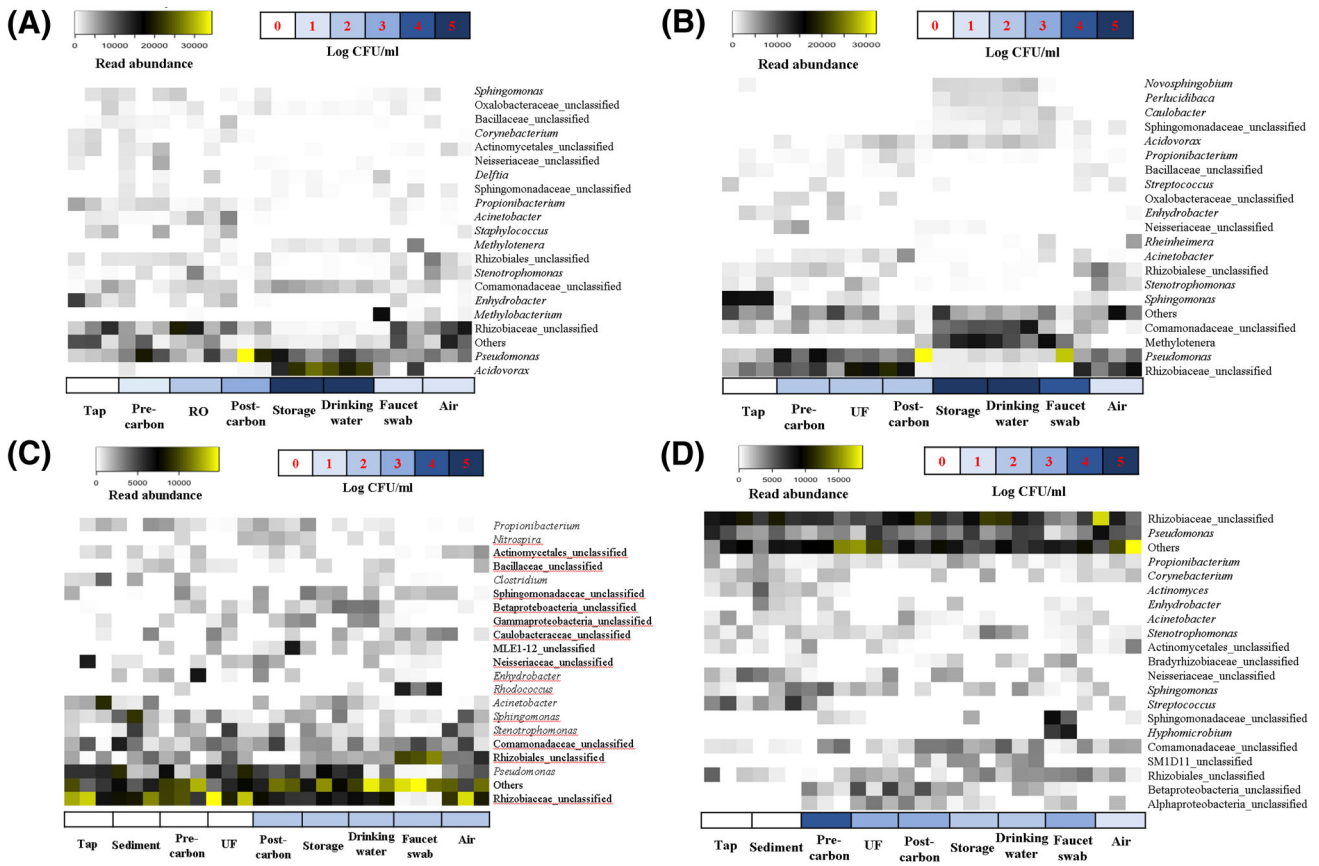

Fig. 1 Genus-level comparison for dispensers A–D (**A**)–(**D**). Gradated blue indicates Log_{10} CFU/ml

of dispenser D, but it decreased during the subsequent membrane filtration steps. Further study is needed to understand about the increase in bacterial count in the pre-carbon filter.

Our results only suggest that the abundance of *Sphingomonas*, a ubiquitous freshwater bacterium, and an unknown genus affiliated to the family Comamonadaceae increased. Faucet surface swab HPCs were higher in dispenser B and D samples. The dispenser B swab samples comprised *Pseudomonas*, *Methylotenera*, and unclassified genera belonging to the families Comamonadaceae and Rhizobiaceae that were also found prevalent in drinking water. Dispenser D swab samples mostly comprised *Hyphomicrobium,* known general oral bacteria [18] and were detected neither in water samples nor in air samples, suggesting that the bacteria were transferred from cups and an unclassified genus belonging to the family Sphingomonadaceae.

Microbial community comparison based on OTUs

After normalizing the data size, 39,328 reads per sample were obtained. Minimum coverage was higher than 0.999 (data not shown), and number of OTUs per sample ranged from 17 to 127. Differential abundance test showed no significant difference between tap water and air microbial communities (data not shown). Results shown in Fig. 2 indicated clear separations between water samples before and after filtration for the basement dispensers A and B (cluster I and II), while only faucet surface swabs showed similar separations in water samples from dispenser C and D (cluster I and II). Differential abundance test between clusters was performed, and the results are summarized in Table 1. The basement dispensers A and B showed a significant increase in the abundance of the freshwater bacteria *Acidovorax* and *Methylotenera* ($P < 0.05$) in the water storage although these bacteria were hardly found at the filtration steps and in the air. Therefore, the source of these bacteria is likely from the tap water and removal of the disinfectant residue allowed them to proliferate in the storage tank. Dispenser C showed significant increase in the abundance of *Rhodococcus* and *Mycoplana,* both of which are ubiquitous environmental bacteria, on the faucet surface. Dispenser D showed significant increase in the abundance of *Hyphomicrobium* and of an unknown genus belonging to the family Rhizobiaceae and Bradyrhizobiaceae. As aforementioned, *Hyphomicrobium* is an oral

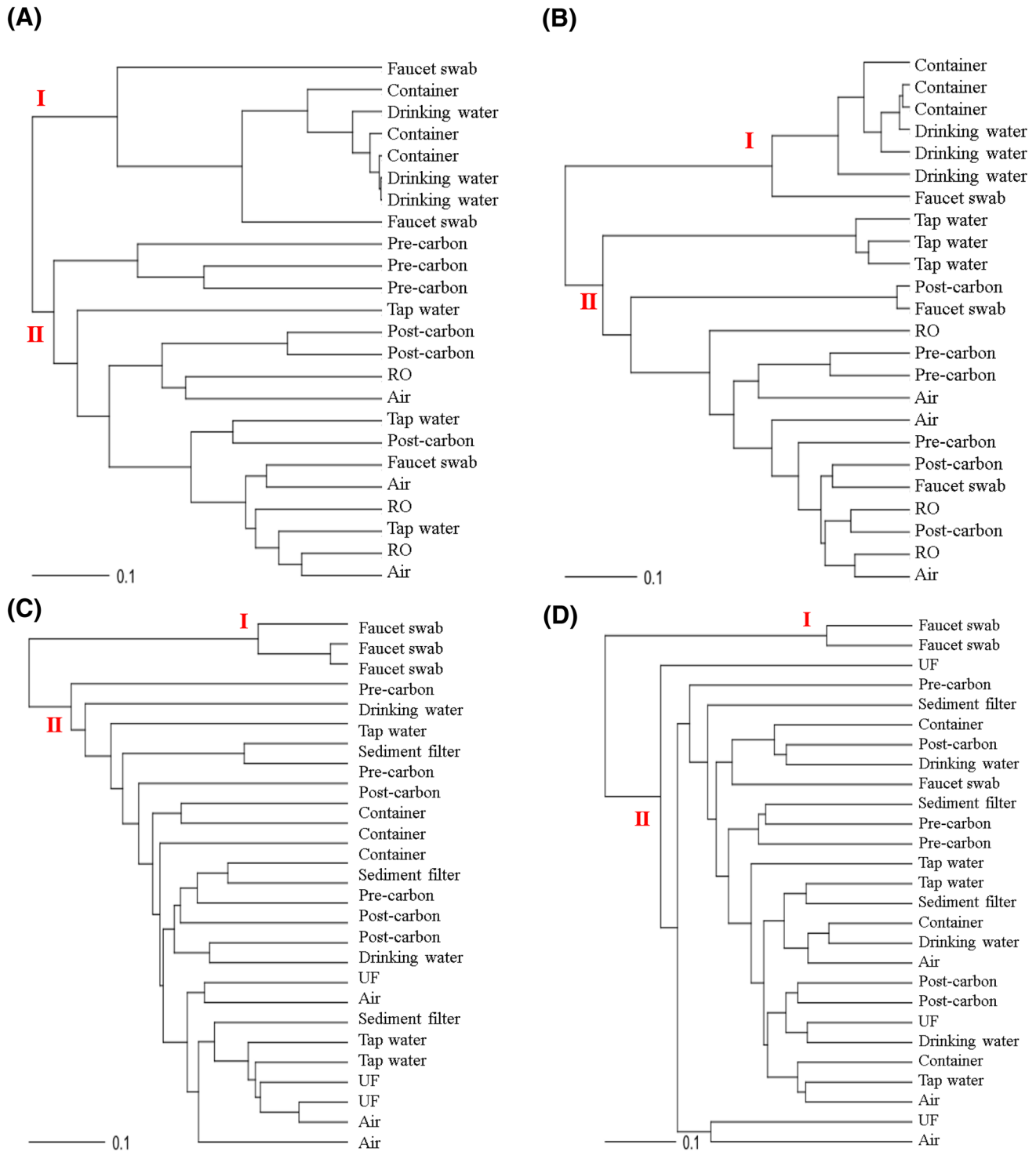

Fig. 2 Cluster analysis of microbial communities obtained from each dispenser A–D (**A**)–(**D**)

bacterium, and the dispenser D faucet may be contaminated with bacteria transferred from cups.

HPC analysis is often used to investigate the microbial quality of water; however, our results showed that taxonomical classification of the bacteria also helps understand the potential sources of bacteria. In this study, we observed higher HPCs in the drinking water stored in nonused dispensers, suggesting that waterborne bacteria may proliferate if stored water is not circulated. However, frequently used dispensers may show higher HPCs around the faucet owing to externally transferred bacteria. Our results also suggest that freshwater bacteria do not proliferate if

Table 1 Top 5 LDA effect size in each cluster shown in Fig. 2

Dispenser	Genus	Cluster	LDA	*P* value
A	*Acidovorax*	I	5.34563	7.23E−05
	Rhizobiaceae_unclassified	II	4.96302	0.000238563
	Methylotenera	I	4.42092	0.000108264
	Comamonadaceae_unclassified	I	4.3758	0.0169048
	Enhydrobacter	II	4.37167	0.0339083
B	*Methylotenera*	I	5.15665	0.000114703
	Rhizobiaceae_unclassified	II	5.06483	0.00255492
	Pseudomonas	II	5.02062	0.0002029
	Comamonadaceae_unclassified	I	4.92543	0.000332769
	Acidovorax	I	4.40189	0.00292055
C	Rhizobiaceae_unclassified	II	5.04516	0.00547855
	Rhizobiales_unclassified	I	5.03693	0.00547141
	Rhodococcus	I	4.80116	0.000580652
	Pseudomonas	II	4.76023	0.00547855
	Mycoplana	I	4.40903	3.61E−07
D	*Hyphomicrobium*	I	4.97197	0.00253917
	Sphingomonadaceae_unclassified	I	4.92751	0.0181454
	Rhizobiaceae_unclassified	II	4.79797	0.0206376
	Pseudomonas	II	4.60482	0.0332223
	Bradyrhizobiaceae_unclassified	I	4.50234	0.00932028

disinfectant residues are not removed. Together, our results suggested that HPC and next generation sequencing (NGS)-based microbial community analyses provide a better understanding of water dispenser microbial contamination than using HPC analysis alone. Further study may include various environmental factors such as temperature and light conditions to determine critical factors that affect microbial distribution in water dispensers in general.

Acknowledgments This study was conducted as a project of National Institute of Environmental Research funded by the Korean government (1900-1946-303-210).

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