

Molecular characterization of natural biofilms from household taps with different materials: PVC, stainless steel, and cast iron in drinking water distribution system

Wenfang Lin · Zhisheng Yu · Xi Chen · Ruyin Liu ·
Hongxun Zhang

Received: 3 September 2012 / Revised: 25 October 2012 / Accepted: 27 October 2012 / Published online: 10 November 2012
© Springer-Verlag Berlin Heidelberg 2012

Abstract Microorganism in drinking water distribution system may colonize in biofilms. Bacterial 16S rRNA gene diversities were analyzed in both water and biofilms grown on taps with three different materials (polyvinyl chloride (PVC), stainless steel, and cast iron) from a local drinking water distribution system. In total, five clone libraries (440 sequences) were obtained. The taxonomic composition of the microbial communities was found to be dominated by members of Proteobacteria (65.9–98.9 %), broadly distributed among the classes Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria. Other bacterial groups included Firmicutes, Acidobacteria, Bacteroidetes, Cyanobacteria, and Deinococcus-Thermus. Moreover, a small proportion of unclassified bacteria (3.5–10.6 %) were also found. This investigation revealed that the bacterial communities in biofilms appeared much more diversified than expected and more care should be taken to the taps with high bacterial diversity. Also,

regular monitor of outflow water would be useful as potentially pathogenic bacteria were detected. In addition, microbial richness and diversity in taps ranked in the order as: PVC < stainless steel < cast iron. All the results interpreted that PVC would be a potentially suitable material for use as tap component in drinking water distribution system.

Keywords Biofilms · Tap materials · Drinking water distribution · 16S rRNA gene

Introduction

Drinking water is distributed through complicated pipe systems until it arrives at consumers' tap (Lee et al. 2010). As taps are one of the endpoints in the drinking water distribution, citizens are exposed to and consume large amounts of tap water in their daily life (Von Baum et al. 2010). However, drinking water quality is not monitored routinely at the household level but rather directly in the distribution system, as waterworks and authorities have limited access to private homes, which increases drinking water risk (Lautenschlager et al. 2010). Even though the drinking water distribution system is well treated and strictly managed, both water and biofilms may still harbor microorganisms (Lehtola et al. 2004). It has been estimated that about 95 % of all microbial cells present in drinking water distribution systems exist as biofilms on pipe surfaces and only 5 % occur in the water phase (Moritz et al. 2010).

Microbial growth in the drinking water distribution system leads to a deterioration of aesthetic water quality like undesirable tastes, odors, and visual turbidity, and even pipe corrosion (Hammes et al. 2008). In addition, the occurrence of biofilms may also cause growth of potentially pathogenic

W. Lin · Z. Yu (✉) · R. Liu · H. Zhang
College of Resources and Environment,
University of Chinese Academy of Sciences,
19 A. Yuquan Road,
100049, Beijing, Shijingshan District, People's Republic of China
e-mail: Yuzs@gucas.ac.cn

Z. Yu
Department of Engineering Science, University of Oxford,
Parks Road,
OX1 3PJ, Oxford, UK

X. Chen
Advanced Environmental Biotechnology Centre,
Nanyang Technological University,
639798, Singapore, Singapore

X. Chen
School of Biological Sciences, Nanyang Technological University,
639798, Singapore, Singapore

bacteria (Lehtola et al. 2007; Giao et al. 2008; Wingender and Flemming 2011). Potentially pathogenic bacteria such as *Legionella* and *Mycobacteria* may easily cause a potential health risk, especially for immunocompromised individuals, when contaminated water pass through taps and aerosolize in indoor air (Hedayati et al. 2011). Moreover, it is well-known that bacteria in biofilms display a much higher tolerance to the environment than suspended counterparts (Flemming and Ridgway 2009). Hence, to be more efficient in the control of biofilms, a better understanding of the mechanisms involved in the biofilm development, especially those factors affecting the rate of bacterial colonization and regrowth, is necessary (Momba and Makala 2004). There are several factors which can influence the formation of biofilm, e.g., nutrients, disinfectant residual concentration, water hydraulic conditions, and materials (Momba et al. 2000; Lehtola et al. 2002). The most complicated effects on biofilm formation come from attached materials.

Many studies have focused on the effect of pipe materials on biofilm formation, which makes up drinking water distribution systems. For instance, (Kerr et al. 1999) compared biofilm accumulation and heterotrophic bacterial diversity on three pipe materials, cast iron, medium density polyethylene (MDPE), and unplasticized polyvinyl chloride (uPVC), and found that MDPE and uPVC supported the lowest number of bacteria, but the diversity of heterotrophic bacteria was greatest on cast iron. In contrast to the study, research work carried out by (Paquin et al. 1992) reported that the number of heterotrophic plate count of the biofilm grown on three types of coupons (polyvinyl chloride (PVC), polyethylene (PE), and cement-lined cast iron) did not show any difference. Therefore, there is still controversy about the effect of surface materials on biofilm development. Based on the previous studies on bacteria–surface material interaction, we wonder about the relationship between bacterial communities and attached tap materials. To our knowledge, domestic tap materials have been considered less frequently (Rudi et al. 2009). Therefore, more research is required in this field. In order to study drinking water biofilms, researchers have traditionally used culturing methods. However, only a small proportion of microorganisms can be studied using culture techniques (Phung et al. 2004; Roeder et al. 2010). Hence, molecular methods have been developed using 16S rRNA gene-based approaches to identify bacterial species and assess their abundances within drinking water communities (Henne et al. 2012).

The aim of the present study was to observe the microbe changes occurring in inflow and outflow water samples through the network and investigate bacterial community on taps with three different materials (PVC, stainless steel, and cast iron) of a more than 10-year-old drinking water network using 16S rRNA gene clone libraries.

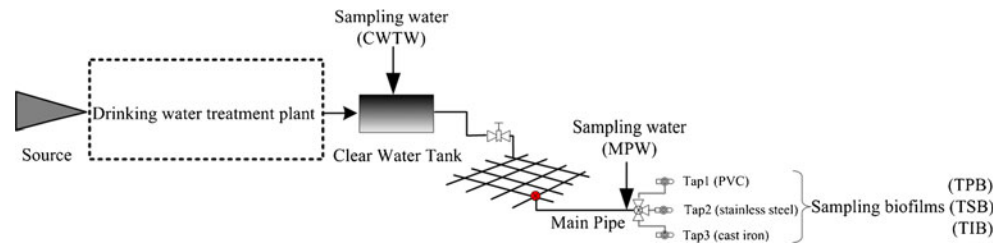
Materials and methods

Water and biofilm sampling

The water and biofilm samples were analyzed from a drinking water distribution system of Hubei Province, China. The source water entered the drinking water treatment plant and went through various treatment procedures including coagulation–flocculation, settling, and filtration. A final disinfection step to produce finished drinking water was carried out by the addition of ClO_2 in the clear water tank. Also, free residual chlorine was subsequently maintained in the drinking water distribution system. The drinking water was delivered to the consumer endpoints such as taps by the stainless steel main pipes (Fig. 1). Three taps with different materials: PVC, stainless steel, and cast iron have been installed in the kitchen of one household since 2001, and all frequently provided cold water for the consumers by high usage from 6 a.m. to 10 p.m. every day. Consequently, mature biofilms were established on the inner surface of the taps. In addition, the household was located about 0.8 km from the drinking water treatment plant.

Five samples were obtained from the drinking water distribution system, which were the inflow water in the clear water tank (CWTW), the outflow water in the main pipe (MPW) shared with the three taps, as well as the biofilms in the taps with three materials: PVC (TPB), stainless steel (TSB), and cast iron (TIB). Water samples were collected in the clear water tank and the main pipe, respectively, using sterile 20-L carboys. Prior to sampling the outflow water (MPW), the tap was run for 10 min to remove standing water in the pipes to obtain representative samples. When sampling, some water quality parameters including temperature, pH, and total dissolved solid were measured using YSI Professional Plus (YSI Incorporated, USA) and free residual chlorine was tested with colorimetry of orthotolidine in situ. Some other parameters including turbidity, COD_{Mn} , and nitrate and total plate count were sent to the Pony Testing International Group (Beijing, China) and strictly measured with the standard methods (GB: 5749–2006) in China. A volume of 5 L for each water samples was filtered with 0.22- μm polycarbonate membranes (47 mm diameter, Millipore, MA, USA) to get the microorganisms. Taps with different materials (PVC, stainless steel, and cast iron) were removed to collect the biofilms on them. Photographs were taken using a digital camera (Canon IXUS 220 HS, Japan) to show their shapes (Fig. 2). Both of the filter membranes and the biofilm samples were kept in an ice box during transportation. After reaching the laboratory, the outside surfaces of the taps were rinsed and cleaned carefully using sterile water. Before processing, the tap handles were turned off. Then, the biofilms on the taps were scraped out from the inner

Fig. 1 Schematic diagram of the drinking water distribution system used in this study



surfaces (15 mm inside; 10 cm long) at the connection point with the pipe line using a sterile scraper and then suspended in 5 mL sterile water. The biofilm suspensions were then centrifuged at 10,000g for 10 min. The pellets were resuspended in 1 mL sterile water in 2-mL tubes (Emtiazi et al. 2004). Finally, all samples were stored at -70°C before usage.

Nucleic acid extraction, cloning, and sequencing of 16S rRNA genes

The filter membranes were aseptically torn into several pieces with forceps and then placed into 2-mL tubes. DNA was extracted using the commercial FastDNA SPIN Kit (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's instructions (Roeder et al. 2010). The extracted nucleic acids were visualized by electrophoresis in a 0.8 % agarose gel in TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM ethylene diamine tetraacetic acid (EDTA), pH 8.3) and kept at -70°C until use. These DNA preparations were used as template in polymerase chain reaction (PCR).

Bacterial 16S rRNA genes were amplified by the PCR using the forward primer 27 F (5' AGAGTTTGATCCTGGCTCAG 3') and the reverse primer 1492R (5' GGTTACCTTGT TACGACTT 3'). Reactions were performed in a total volume of 50 μL comprising 200 μM of each dNTP, 0.2 μM of each primer, 10 \times PCR Buffer, and 2.5 U of *Taq* polymerase (Takara Mirus Bio Corp., Madison, Japan). DNA templates were diluted four times and then added into the reaction. The following conditions were used for DNA amplification: 5 min at 94°C , followed by 35 cycles of 45 s at 94°C , 45 s at 56°C , and 1 min 30 s at 72°C , and a final extension at 72°C for 10 min. In addition, reactions containing sterile water (no template) served as negative DNA control. Products were screened by gel electrophoresis in 1 % agarose gel.

All PCR products were further purified with the PCR purification Kit (Fermentas, USA) following the manufac-

turer's instructions. About 1.5-kb long fragments were ligated with 3 U of T4 DNA ligase and 1 \times T4 DNA ligase buffer overnight at 4°C . Cloning reactions were carried out as follows: transformed *Escherichia coli* (Takara Mirus Bio Corp., Madison, Japan) were spread-plated onto LB agar containing 100 $\mu\text{g}/\text{mL}$ ampicillin and incubated overnight at 37°C (Williams et al. 2004). Selected colonies were screened by carrying out PCR amplification with M13 forward primer (5' GTTTTCCCAGTCACGAC 3') and M13 reverse primer (5' CAGGAAACAGCTATGAC 3'), followed by gel electrophoresis on 1 % agarose gel at 180 V for 20 min. The positive clones were incubated in LB broth containing 100 $\mu\text{g}/\text{mL}$ ampicillin for 12 h and then sent to Majorbio Bio-pharm Technology Co., Ltd., China for sequencing.

Phylogenetic and statistical analyses

Sequencing reads were trimmed of vector and inspected for ambiguities using Sequencher 4.1 software (Gene Codes, Ann Arbor, MI, USA). All 16S rRNA gene sequences were examined for chimeras with the Mallard program version 1.02 and screened for putative anomalies (Ashelford et al. 2006). The 16S rRNA gene sequences were affiliated to bacterial taxa using SeqMatch on the RDP (Ribosomal Database Project) website. Multiple sequence alignment and clustering into Operational Taxonomic Units (OTUs) were performed with mothur (<http://www.mothur.org>), using a 97 % similarity cutoff. Representative clones were selected from each OTU and compared with available rRNA gene sequences in GenBank (<http://www.ncbi.nlm.nih.gov/>) using the NCBI Blast program. Mothur was also used to generate rarefaction curve and community tree and to calculate richness estimators and diversity index including abundance-based coverage estimator (ACE), Chao 1 and Shannon's and Simpson's indices. The coverage of clone libraries was calculated according to the reference (Moissl

Fig. 2 Tap samples with three different materials: PVC (a), stainless steel (b), and cast iron (c) taken from one household



et al. 2007) using the formula $[1 - (ni / N)]$, where ni is the number of OTUs represented by only one clone (singletons) and N is the total number of the clones examined in each library]. Additionally, the shared OTUs were used to estimate similarity between communities based on membership and structure. For further downstream analysis, Origin7.0 (OriginLab Corporation) was used to draw the figs. The 16SrRNA gene sequences determined in this study are available in the GenBank database under accession numbers JQ923506 to JQ924029.

Results

Water quality analysis

The standard water chemistry and bacteriology data at the time of sampling are summarized in Table 1. All the physical and chemical qualities of drinking water met the national drinking water standard (GB: 5749–2006) in China. The results revealed that total plate count (nutrient agar, 37 °C, 48 h), COD_{Mn} , and NO_3^- -N slightly increased during transportation. However, free residual chlorine concentration became low, about 20 % of the initial concentration.

Sequence diversity analysis

A total of 440 partial 16S rRNA gene sequences were analyzed in this study. The sequences were obtained from five different clone libraries representing five different samples: CWTW (81 clones), MPW (86 clones), TPB (85 clones), TSB (85 clones), and TIB (103 clones). Diversity analysis including rarefaction curves, community tree, richness estimators, and diversity indices was used to assess the level of richness, evenness, and community membership of the clones using 97 % as the taxonomic unit cutoff (Table 2). The number of observed OTUs for the five samples was

different (CWTW=23; MPW=20; TPB=17; TSB=28; TIB=37). The expected OTUs (using Chao 1) were higher than the observed OTUs (CWTW=36; MPW=98; TPB=20; TSB=43; TIB=83), suggesting a large degree of bacterial diversity for these five samples. The coverage values (>78 %) confirmed that the core communities were covered in all samples. These results were consistent with comparisons of richness based on calculations using rarefaction curve (Fig. 3). Apparent differences of bacterial diversity were observed between inflow water (CWTW) and outflow water (MPW). The species richness estimators (Chao1 and ACE) showed MPW had higher diversity than CWTW. Metallic materials (TSB and TIB) supported higher diversity than plastic ones (TPB). When OTU pairwise comparisons were performed, it was found that TSB and TIB shared a higher number of OTUs (four shared OTUs) than TPB and TSB (two shared OTUs), and TPB and TIB (two shared OTUs). The number of OTUs shared between groups TPB, TSB, and TIB is one OTU (Venn diagram data not shown). Finally, community tree (Fig. 4) analysis was used to show the similarity of different samples. Clone libraries from two metallic taps resembled each other and clustered together. However, sample TPB clustered with samples CWTW and MPW.

Microbial community analysis of drinking water and biofilms

Community profiles were prepared, using these assignments, for each sample (Fig. 5). Proteobacteria represented the majority of microbes within the bacteria community, accounting for 96.3 %, 98.9 %, 91.8 %, 95.3 %, and 87.4 % for CWTW, MPW, TPB, TSB, and TIB, respectively. The Proteobacteria were affiliated to the classes Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria, as well as some unclassified Proteobacteria. Differences in the relative abundance of Proteobacteria classes were observed in the two water samples. For example, Alphaproteobacteria were more abundant in MPW (66.7 %) than in CWTW (27.5 %). In contrast, Beta- (i.e., 20.6 % vs. 30 %) and Gammaproteobacteria (i.e., 11.5 % vs. 38.8 %) were less abundant in MPW as compared to CWTW. The analysis of the microbial community structures in the three tap biofilms suggested that TSB sample was similar to TIB sample, whereas TPB sample was different from TSB and TIB samples. Betaproteobacteria sequences predominated in TSB (56.5 %) and TIB (47.6 %) clone libraries, while these did not appear in TPB clone library. Further evaluation at the order level (Fig. 6) showed that Alphaproteobacteria represented by Sphingomonadales and Rhizobiales in all samples. Moreover, Burkholderiales order was found to be predominant within the Betaproteobacteria. Within the Gammaproteobacteria class, bacterial groups comprised

Table 1 Summary of water quality at the time of sampling

Chemical and physical characteristics	CWTW	MPW
Turbidity (NTU)	<0.50	<0.50
pH value	7.36	7.46
Nitrate (mg/L, as NO_3)	1.54	1.56
Chloride (mg/L, as ClO_2)	0.10	0.02
Total plate count (CFU/mL)	ND	28
Total dissolved solid (mg/L)	0.24	0.25
Temperature(°C)	23.18	24.32
COD_{Mn} (mg/L)	0.96	1.03

ND not detected, CWTW water from clear water tank, MPW water from main pipe

Table 2 Sequence diversity and richness estimators

	CWTW	MPW	TPB	TSB	TIB
Number of sequences	81	86	85	85	103
Number of OTUs ^a	23	20	17	28	37
Singletons	12	13	6	16	22
Chao 1 estimator of OTU richness	36	98	20	43	83
ACE estimator of OTU richness	55	81	28	86	149
Shannon index of diversity	2.54	2.09	2.11	2.49	3.15
Simpson index of diversity	0.11	0.22	0.19	0.17	0.05
Rate of coverage (%)	85	85	93	81	79

^aOTUs were defined at 97 % sequence similarity cutoff level, OTUs operational taxonomic units, ACE abundance-based coverage estimator, CWTW water from clear water tank, MPW water from main pipe, TPB biofilms on tap of PVC, TSB biofilms on tap of stainless steel, TIB biofilms on tap of cast iron

potential pathogen species, including Legionellales, Enterobacteriales, and Pseudomonadales orders. Among these, the biofilms were tested for the presence of *Legionella* spp. at the samples TPB, TSB, and TIB. The Enterobacteriales were only found at the sample TSB. Additionally, the Pseudomonadales were detected at all samples. Other bacteria included Firmicutes, Acidobacteria, Bacteroidetes, Cyanobacteria, and Deinococcus-Thermus. One clone related to Deinococcus-Thermus was found merely in the CWTW sample. Similarly, one clone that showed high sequence identity (99 %) with Cyanobacteria was barely detected in MPW sample. Also, it was quite evident from this work that bacterial biofilms in TIB sample harbored a diverse group of bacteria. Within Gammaproteobacteria, 11 clones showed 87 % similarity with the Legionellales; thus, we could not exactly confirm them. Acidobacteria and Bacteroidetes were found in TIB samples. Additionally, it was interesting to note that a small proportion of sequences were identified as belonging to unclassified bacteria from TPB (3.5 %) and TIB (10.6 %), respectively. It also should be noted that 17 clones showed

high sequence identity (97 %) with iron-reducing bacterium (FJ269052) in TIB samples.

Discussion

There have been numerous studies that showed that biofilms can form on the surface of pipelines and revealed the differences among the materials including biofilm formation capacity, fixed bacterial biomass, and morphology feature (Niquette et al. 2000). This study represented a molecular survey using 16S rRNA clone libraries on water and biofilms to describe the microbial communities. In particular, the taps with three different materials have been used for more than 10 years. The biofilms grown on them should be very stable and mature, supplied with the same water source, treated by the same processes, and located in one household with analogous conditions.

Analysis of bacterial diversity suggested that MPW had higher diversity than CWTW. The reason might be involved with the biofilms' growth on the surfaces of pipelines and free chlorine loss in distribution system, which was consistent with variational water quality such as increased total plate count and lower free chlorine (Bachmann and Edyvean 2005; Al-Jasser 2007; Zhang et al. 2008). Comparing TSB and TIB to TPB, higher shared OTUs were retrieved from both metallic materials (stainless steel and cast iron). Moreover, cluster analysis of the population profiles, samples TSB and TIB cluster together and samples TPB,

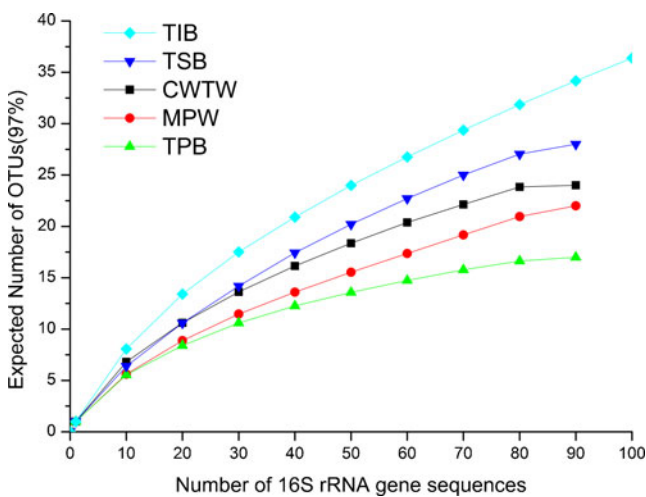


Fig. 3 Rarefaction curves of OTUs based on a similarity threshold of 97 %. CWTW water from clear water tank, MPW water from main pipe, TPB biofilms on tap of PVC, TSB biofilms on tap of stainless steel, TIB biofilms on tap of cast iron

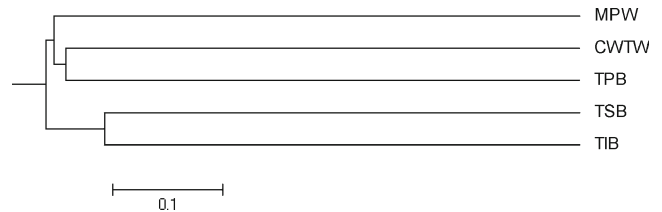
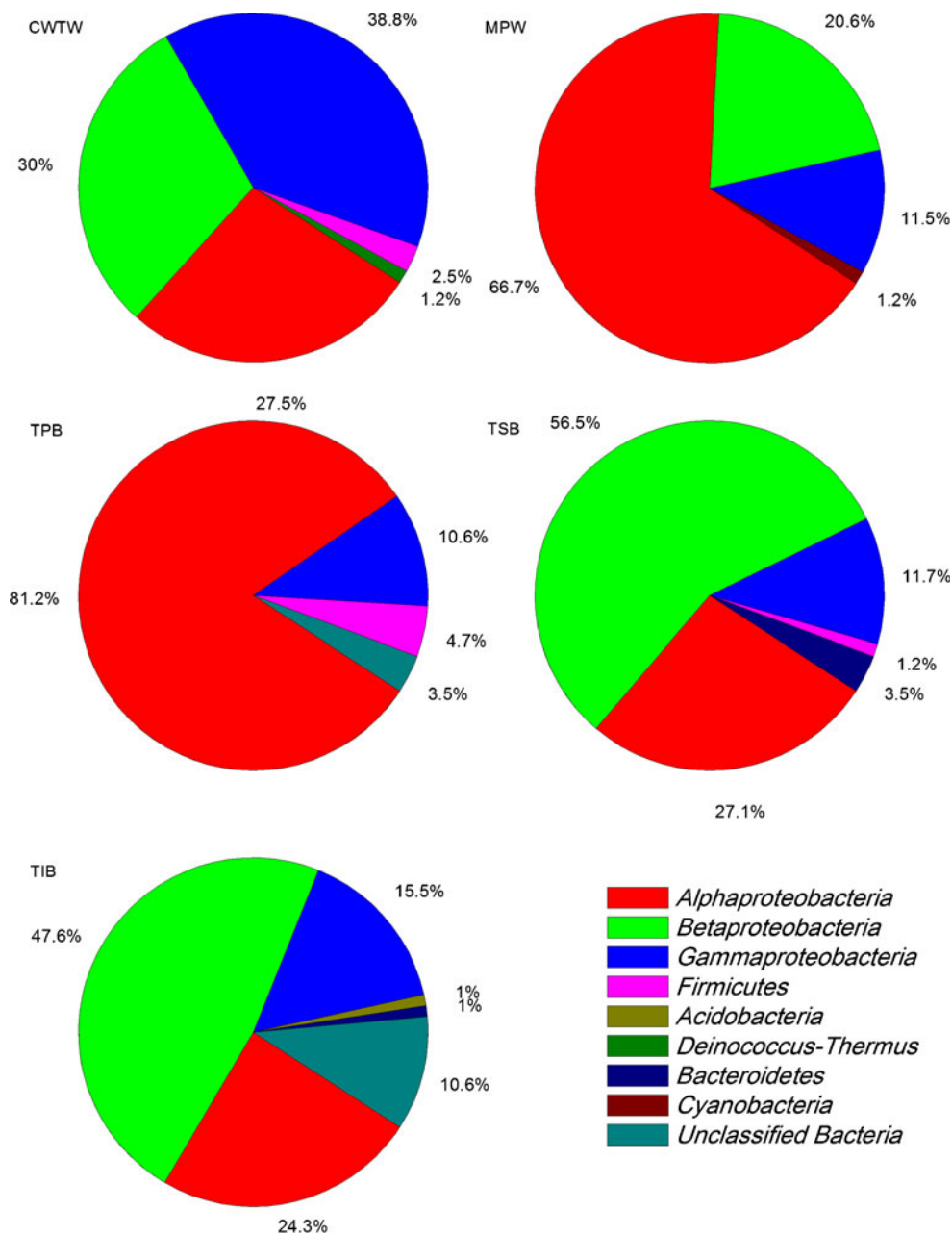


Fig. 4 Cluster analysis of the population profiles from water and biofilm samples to show their relationships according to the Yue and Clayton theta structural diversity measure. CWTW water from clear water tank, MPW water from main pipe, TPB biofilms on tap of PVC, TSB biofilms on tap of stainless steel, TIB biofilms on tap of cast iron

Fig. 5 Microbial composition of water and biofilms in drinking water distribution determined by 16S rRNA gene sequence analysis. *CWTW* water from clear water tank, *MPW* water from main pipe, *TPB* biofilms on tap of PVC, *TSB* biofilms on tap of stainless steel, *TIB* biofilms on tap of cast iron



CWTW, and CWTB cluster together. These results suggested that bacterial community seems dependent on the tap materials. However, it has been shown that sample TPB clustered with samples CWTW and MPW, which might be explained by bacteria transmitted from the clear water tank to consumer taps during drinking water distribution.

Our results showed that the microbial communities in all samples consisted principally of Proteobacteria. This is in good agreement with earlier studies from drinking water systems, suggesting that these organisms were well suited to survive in potable water supplies (Kormas et al. 2010; Revetta et al. 2011). When comparing samples of MPW to CWTW, the Alphaproteobacteria increased from 27.5 % to

66.7 %. Beta- and Gammaproteobacteria decreased from 30 % to 20.6 % and 38.8 % to 11.5 %, respectively. Differences in the Proteobacteria percentages within our drinking water might be explained by free chlorine concentration changes during distribution. The proportion of Alphaproteobacteria declined when chlorine concentrations increased (Mathieu et al. 2009). One unexpected finding, however, is the striking lack of Betaproteobacteria among our identified TPB clone library, given that members of this division are usually abundant in freshwaters as well (Manz et al. 1999; MacDonald and Brozel 2000). The reason for this apparent absence is unknown, but it might be related to the habitat characteristic, particularly attached materials (Buesing et al. 2009). (Schwartz et al. 1998) suggested that

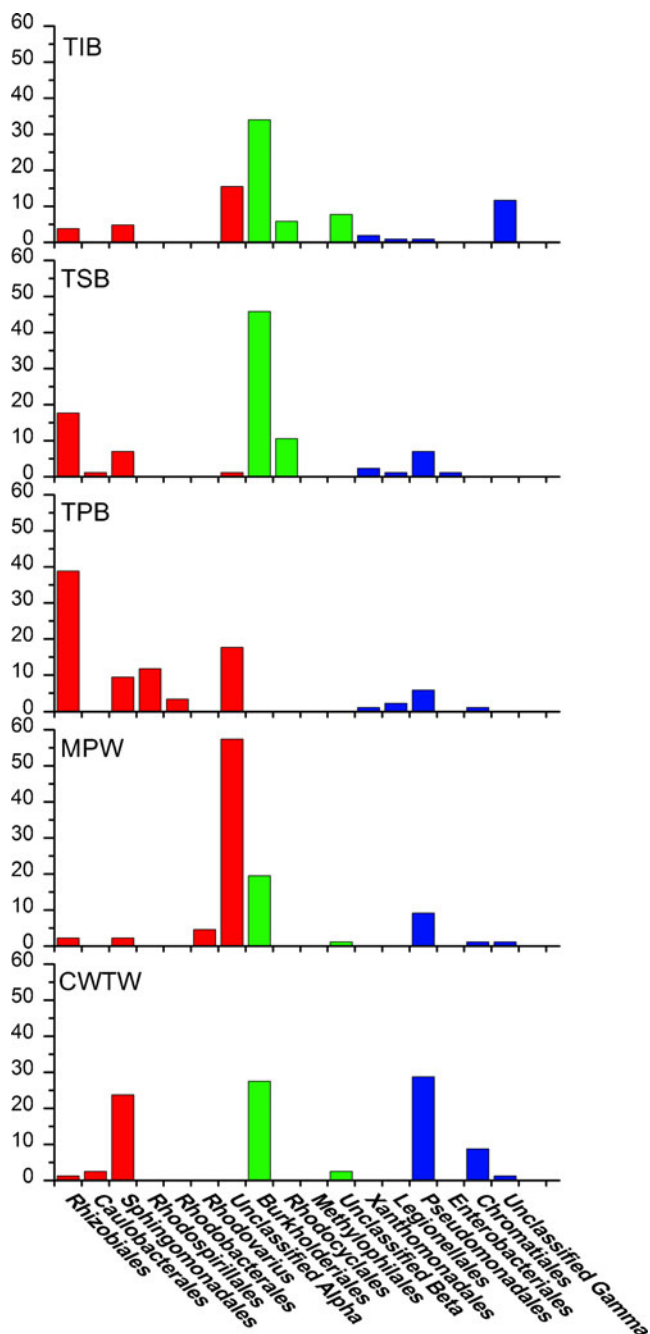


Fig. 6 The taxonomic distribution of Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria and Deltaproteobacteria orders for each sample. *CWTW* water from clear water tank, *MPW* water from main pipe, *TPB* biofilms on tap of PVC, *TSB* biofilms on tap of stainless steel, *TIB* biofilms on tap of cast iron

plastic materials such as PVC, widely used in domestic drinking water distribution systems, appeared to be colonized very frequently by Beta- and Gammaproteobacteria, whereas the Beta subclass was detected in higher percentages on metallic materials (steel and copper).

In addition, environmental strains such as Rhizobiales were commonly found in soil environments, and it was

suspected that they were initially present in the water sources (Poitelon et al. 2009). The *Sphingomonas* belonging to Alphaproteobacteria were isolates from water distribution system tap water, and it is known that the species can survive chlorination by forming bacterial aggregates (Yu et al. 2010). It is intriguing to find Cyanobacteria population detected in MPW when the source of water was surface water. Though most of these organisms depend on light for survival, it was possible that some of the Cyanobacteria populations detected in this study can temporarily survive the darkness under anaerobic conditions (Revetta et al. 2010). Recent studies have shown that the Bacteroidetes are frequently isolated in aquatic environments (Poitelon et al. 2010). Other minor sequences closely related to Acidobacteria and Firmicutes have recently been found in a distribution network. In general, these lineages are widely detected in terrestrial environments or other locations. This suggests that microorganisms might take root in the aquifer supplying the distribution network (Martiny et al. 2005). Nonetheless, the significance of the bacterial groups is not currently discernible and requires much further research.

Molecular analysis of microbial communities clearly showed that the microbial richness and diversity are significantly related to the pipe materials by the order: PVC (TPB) < stainless steel (TSB) < cast iron (TIB). There are some reasons for it. Initially, microbial adhesion to surfaces is a complex process influenced by several physicochemical properties of both microorganism and substratum. Strongly adherent bacteria may play a determinant role in the primary colonization of surfaces, seeding biofilms which will subsequently develop by cellular proliferation and immigration (Simoes et al. 2007). For example, *Sphingomonas* and other biofilm-associated slime producers, like Rhizobiales bacteria are responsible for bacteria colonization (Pang and Liu 2007). Furthermore, the influence that attached materials exert on bacterial adhesion and biofilm formation is rooted in characteristics such as surface structure and chemical composition (Waines et al. 2011). For instance, roughness of pipe surface and corrosion products affects bacterial attachment on the surface. Hence, biofilm regrowth on pipes made of rough surface materials such as cast iron and galvanized steel was greater than that on smooth surface PVC pipe (Yu et al. 2010; Chowdhury 2011). The stainless steels, as an alloy metal, depend for their corrosion resistance on a thin film, but it may corrode when environmental conditions become aggressive enough to take advantage of the weakness in the film (Percival et al. 1997; Percival et al. 1998). In addition, pipe corrosion contributed to the bacterial regrowth in a distribution system. Conversely, biofilm can also promote corrosion of metals (Teng et al. 2008). Bacteria identified on corroding surfaces encompass a range of species, including sulfate-reducing bacteria (SRB),

sulfur-oxidizing bacteria (SOB), and bacteria-secreting organic acids and slime (Dong et al. 2011; Wang et al. 2012). Thus, it was not surprising to find that two OTUs (17 clones) in sample TIB was closely related to iron-reducing bacterium (FJ269052). Based on this study, PVC taps supported a lower level of bacterial diversity than the other two materials, which suggested that PVC may be a potentially suitable material for use as a major component in drinking water distribution. The result agreed with previous studies by traditional culture technology showing that plastic-based material supports less fixed biomass than metal materials (Kerr et al. 1999; Niquette et al. 2000). Finally, to explore the impact of attached materials on biofilms, a model experiment will be designed in the next paper.

The occurrence of pathogenic bacteria in the drinking water distribution environment has been reported previously (Felfoldi et al. 2010; Wingender and Flemming 2011). In this study, pathogenic bacteria, like Legionellales, Enterobacteriales, and Pseudomonadales, were detected. However, it should be noted that it is impossible to determine if the bacteria that we identified represented dead or live cells especially for the pathogens. So the significance of the bacterial groups detected in this study clearly requires much further research. Moreover, in recent years, researchers have reported that amoebas could act as vehicles for multiplication and shelter from antibiotic and disinfection treatments (Hsu et al. 2009; Lau and Ashbolt 2009). Our continuing objective is to elucidate the active members of drinking water microbial communities via reverse transcriptase (RT)-PCR, particularly for pathogen and symbiotic protozoa.

Acknowledgments We express gratitude to Ms. Ping Wang, Mr. Zhimin Yu, and members of the local drinking water plant for helping in sampling. This work was supported by the Knowledge Innovation Program of the Chinese Academy of Sciences (Y025014EA2) and the Natural Science Foundation of China (No.51208501).

References

- Al-Jasser A (2007) Chlorine decay in drinking-water transmission and distribution systems: Pipe service age effect. *Water Res* 41:387–396. doi:10.1016/j.watres.2006.08.032
- Ashelford KE, Chuzhanova NA, Fry JC, Jones AJ, Weightman AJ (2006) New screening software shows that most recent large 16S rRNA gene clone libraries contain chimeras. *Appl Environ Microbiol* 72:5734–5741. doi:10.1128/AEM.00556-06
- Bachmann R, Edyvean R (2005) Biofouling: an historic and contemporary review of its causes, consequences and control in drinking water distribution systems. *Biofilms* 2:197–227. doi:10.1017/S1479050506001979
- Buesing N, Filippini M, Burgmann H, Gessner MO (2009) Microbial communities in contrasting freshwater marsh microhabitats. *FEMS Microbiol Ecol* 69:84–97. doi:10.1111/j.1574-6941.2009.00692.x
- Chowdhury S (2011) Heterotrophic bacteria in drinking water distribution system: a review. *Environ Monit Assess* 182:1–51. doi:10.1007/s10661-011-2407-x
- Dong ZH, Liu T, Liu HF (2011) Influence of EPS isolated from thermophilic sulphate-reducing bacteria on carbon steel corrosion. *Biofouling* 27:487–495. doi:10.1080/08927014.2011.584369
- Emtiazi F, Schwartz T, Marten SM, Krolla-Sidenstein P, Obst U (2004) Investigation of natural biofilms formed during the production of drinking water from surface water embankment filtration. *Water Res* 38:1197–1206. doi:10.1016/j.watres.2003.10.056
- Felfoldi T, Heeger Z, Vargha M, Marialigeti K (2010) Detection of potentially pathogenic bacteria in the drinking water distribution system of a hospital in Hungary. *Clin Microbiol Infect* 16:89–92. doi:10.1111/j.1469-0691.2009.02795.x
- Flemming HC, Ridgway H (2009) Biofilm control: conventional and alternative approaches. *Mar Ind Biofouling* 4:103–117. doi:10.1007/7142_2008_20
- Giao MS, Azevedo N, Wilks SA, Vieira M, Keevil CW (2008) Persistence of *Helicobacter pylori* in heterotrophic drinking-water biofilms. *Appl Environ Microbiol* 74:5898–5904. doi:10.1128/AEM.00827-08
- Hammes F, Berney M, Wang Y, Vital M, Koster O, Egli T (2008) Flow-cytometric total bacterial cell counts as a descriptive microbiological parameter for drinking water treatment processes. *Water Res* 42:269–277. doi:10.1016/j.watres.2007.07.009
- Hedayati M, Mayahi S, Movahedi M, Shokohi T (2011) Study on fungal flora of tap water as a potential reservoir of fungi in hospitals in Sari city. *Iran J Med Mycol* 21:10–14. doi:10.1016/j.mycmed.2010.12.001
- Henne K, Kahlisch L, Brettar I, Hofle MG (2012) Comparison of structure and composition of bacterial core communities in mature drinking water biofilms and bulk water of a local network. *Appl Environ Microbiol* 78:3530–3538. doi:10.1128/AEM.06373-11
- Hsu BM, Lin CL, Shih FC (2009) Survey of pathogenic free-living amoebae and *Legionella* spp. in mud spring recreation area. *Water Res* 43:2817–2828. doi:10.1016/j.watres.2009.04.002
- Kerr CJ, Osborn KS, Robson GD, Handley PS (1999) The relationship between pipe material and biofilm formation in a laboratory model system. *J Appl Microbiol* 85:29S–38S. doi:10.1111/j.1365-2672.1998.tb05280.x
- Kormas KA, Neofitou C, Pachiadaki M, Koufostathi E (2010) Changes of the bacterial assemblages throughout an urban drinking water distribution system. *Environ Monit Assess* 165:27–38. doi:10.1007/s10661-009-0924-7
- Lau HY, Ashbolt NJ (2009) The role of biofilms and protozoa in *Legionella* pathogenesis: implications for drinking water. *J Appl Microbiol* 107:368–378. doi:10.1111/j.1365-2672.2009.04208.x
- Lautenschlager K, Boon N, Wang Y, Egli T, Hammes F (2010) Overnight stagnation of drinking water in household taps induces microbial growth and changes in community composition. *Water Res* 44:4868–4877. doi:10.1016/j.watres.2010.07.032
- Lee J, Lee C, Hugunin K, Maute C, Dysko R (2010) Bacteria from drinking water supply and their fate in gastrointestinal tracts of germ-free mice: a phylogenetic comparison study. *Water Res* 44:5050–5058. doi:10.1016/j.watres.2010.07.027
- Lehtola MJ, Miettinen IT, Keinänen MM, Kekki TK, Laine O, Hirvonen A, Vartiainen T, Martikainen PJ (2004) Microbiology, chemistry and biofilm development in a pilot drinking water distribution system with copper and plastic pipes. *Water Res* 38:3769–3779. doi:10.1016/j.watres.2004.06.024
- Lehtola MJ, Miettinen IT, Martikainen PJ (2002) Biofilm formation in drinking water affected by low concentrations of phosphorus. *Can J Microbiol* 48:494–499. doi:10.1139/W02-048
- Lehtola MJ, Torvinen E, Kusnetsov J, Pitkanen T, Maunula L, Von Bonsdorff CH, Martikainen PJ, Wilks SA, Keevil CW, Miettinen IT (2007) Survival of *Mycobacterium avium*, *Legionella*

- pneumophila*, *Escherichia coli*, and *Caliciviruses* in drinking water-associated biofilms grown under high-shear turbulent flow. *Appl Environ Microbiol* 73:2854–2859. doi:10.1128/AEM.02916-06
- MacDonald R, Brozel V (2000) Community analysis of bacterial biofilms in a simulated recirculating cooling-water system by fluorescent *in situ* hybridization with rRNA-targeted oligonucleotide probes. *Water Res* 34:2439–2446. doi:10.1016/S0043-1354(99)00409-1
- Manz W, Wendt-Potthoff K, Neu T, Szewzyk U, Lawrence J (1999) Phylogenetic composition, spatial structure, and dynamics of lotic bacterial biofilms investigated by fluorescent *in situ* hybridization and confocal laser scanning microscopy. *Microb Ecol* 37:225–237. doi:10.1007/s002489900148
- Martiny AC, Albrechtsen HJ, Arvin E, Molin S (2005) Identification of bacteria in biofilm and bulk water samples from a nonchlorinated model drinking water distribution system: detection of a large nitrite-oxidizing population associated with *Nitrospira* spp. *Appl Environ Microbiol* 71:8611–8617. doi:10.1128/AEM.71.12.8611-8617.2005
- Mathieu L, Bouteleux C, Fass S, Angel E, Block J (2009) Reversible shift in the *alpha*-, *beta*- and *gamma*-*proteobacteria* populations of drinking water biofilms during discontinuous chlorination. *Water Res* 43:3375–3386. doi:10.1016/j.watres.2009.05.005
- Moissl C, Osman S, La Duc MT, Dekas A, Brodie E, DeSantis T, Venkateswaran K (2007) Molecular bacterial community analysis of clean rooms where spacecraft are assembled. *FEMS Microbiol Ecol* 61:509–521. doi:10.1111/j.1574-6941.2007.00360.x
- Momba M, Kfir R, Venter SN, Cloete TE (2000) Overview of biofilm formation in distribution systems and its impact on the deterioration of water quality. *Water SA* 26:59–66
- Momba MNB, Makala N (2004) Comparing the effect of various pipe materials on biofilm formation in chlorinated and combined chlorine-chloraminated water systems. *Water SA* 30:175–182
- Moritz MM, Flemming HC, Wingender J (2010) Integration of *Pseudomonas aeruginosa* and *Legionella pneumophila* in drinking water biofilms grown on domestic plumbing materials. *Int J Hyg Environ Health* 213:190–197. doi:10.1016/j.ijheh.2010.05.003
- Niquette P, Servais P, Savoir R (2000) Impacts of pipe materials on densities of fixed bacterial biomass in a drinking water distribution system. *Water Res* 34:1952–1956. doi:10.1016/S0043-1354(99)00307-3
- Pang CM, Liu WT (2007) Community structure analysis of reverse osmosis membrane biofilms and the significance of *Rhizobiales* bacteria in biofouling. *Environ Sci Technol* 41:4728–4734. doi:10.1021/es0701614
- Paquin J, Block J, Haudidier K, Hartemann P, Colin F, Miazga J, Levi Y (1992) Effect of chlorine on the bacterial colonisation of a model distribution System. *J Water Sci* 5:399–414
- Percival SL, Beech IB, Edyvean RGJ, Knapp JS, Wales DS (1997) Biofilm development on 304 and 316 stainless steels in a potable water system. *Water Environ J* 11:289–294. doi:10.1111/j.1747-6593.1997.tb00131.x
- Percival SL, Knapp JS, Edyvean R, Wales DS (1998) Biofilm development on stainless steel in mains water. *Water Res* 32:243–253. doi:10.1016/s0043-1354(97)00132-2
- Phung NT, Lee J, Kang KH, Chang IS, Gadd GM, Kim BH (2004) Analysis of microbial diversity in oligotrophic microbial fuel cells using 16S rDNA sequences. *FEMS Microbiol Lett* 233:77–82. doi:10.1016/j.femsle.2004.01.041
- Poitelon JB, Joyeux M, Welt B, Duguet JP, Prestel E, DuBow MS (2010) Variations of bacterial 16S rDNA phylotypes prior to and after chlorination for drinking water production from two surface water treatment plants. *J Ind Microbiol Biotechnol* 37:117–128. doi:10.1007/s10295-009-0653-5
- Poitelon JB, Joyeux M, Welt B, Duguet JP, Prestel E, Lespinet O, DuBow MS (2009) Assessment of phylogenetic diversity of bacterial microflora in drinking water using serial analysis of ribosomal sequence tags. *Water Res* 43:4197–4206. doi:10.1016/j.watres.2009.07.020
- Revetta RP, Matlib RS, Santo Domingo JW (2011) 16S rRNA gene sequence analysis of drinking water using RNA and DNA extracts as targets for clone library development. *Curr Microbiol* 63:1–10. doi:10.1007/s00284-011-9938-9
- Revetta RP, Pemberton A, Lamendella R, Iker B, Santo Domingo JW (2010) Identification of bacterial populations in drinking water using 16S rRNA-based sequence analyses. *Water Res* 44:1353–1360. doi:10.1016/j.watres.2009.11.008
- Roeder RS, Lenz J, Tarne P, Gebel J, Exner M, Szewzyk U (2010) Long-term effects of disinfectants on the community composition of drinking water biofilms. *Int J Hyg Environ Health* 213:183–189. doi:10.1016/j.ijheh.2010.04.007
- Rudi K, Tannas T, Vatn M (2009) Temporal and spatial diversity of the tap water microbiota in a Norwegian hospital. *Appl Environ Microbiol* 75:7855–7857. doi:10.1128/AEM.01174-09
- Schwartz T, Hoffmann S, Obst U (1998) Formation and bacterial composition of young, natural biofilms obtained from public bank-filtered drinking water systems. *Water Res* 32:2787–2797. doi:10.1016/s0043-1354(98)00026-8
- Simoes LC, Simoes M, Oliveira R, Vieira MJ (2007) Potential of the adhesion of bacteria isolated from drinking water to materials. *J Basic Microbiol* 47:174–183. doi:10.1002/jobm.200610224
- Teng F, Guan YT, Zhu WP (2008) Effect of biofilm on cast iron pipe corrosion in drinking water distribution system: corrosion scales characterization and microbial community structure investigation. *Corros Sci* 50:2816–2823. doi:10.1016/j.corsci.2008.07.008
- Von Baum H, Bommer M, Forke A, Holz J, Frenz P, Wellinghausen N (2010) Is domestic tap water a risk for infections in neutropenic patients? *Infect* 38:181–186. doi:10.1007/s15010-010-0005-4
- Waines PL, Moate R, Moody AJ, Allen M, Bradley G (2011) The effect of material choice on biofilm formation in a model warm water distribution system. *Biofouling* 27:1161–1174. doi:10.1080/08927014.2011.636807
- Wang H, Hu C, Hu X, Yang M, Qu J (2012) Effects of disinfectant and biofilm on the corrosion of cast iron pipes in a reclaimed water distribution system. *Water Res* 46:1070–1078. doi:10.1016/j.watres.2011.12.001
- Williams M, Domingo J, Meckes M, Kelty C, Rochon H (2004) Phylogenetic diversity of drinking water bacteria in a distribution system simulator. *J Appl Microbiol* 96:954–964. doi:10.1111/j.1365-2672.2004.02229.x
- Wingender J, Flemming HC (2011) Biofilms in drinking water and their role as reservoir for pathogens. *Int J Hyg Environ Health* 214:417–423. doi:10.1016/j.ijheh.2011.05.009
- Yu J, Kim D, Lee T (2010) Microbial diversity in biofilms on water distribution pipes of different materials. *Water Sci Technol* 61:163–171. doi:10.2166/wst.2010.813
- Zhang Z, Stout JE, Yu VL, Vidic R (2008) Effect of pipe corrosion scales on chlorine dioxide consumption in drinking water distribution systems. *Water Res* 42:129–136. doi:10.1016/j.watres.2007.07.054