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ORIGINAL PAPER

Variations of bacterial 16S rDNA phylotypes prior to and after chlorination for drinking water production from two surface water treatment plants

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Abstract We examined the variations of bacterial populations in treated drinking water prior to and after the final chlorine disinfection step at two different surface water treatment plants. For this purpose, the bacterial communities present in treated water were sampled after granular activated carbon (GAC) filtration and chlorine disinfection from two drinking water treatment plants supplying the city of Paris (France). Samples were analyzed after genomic DNA extraction, polymerase chain reaction (PCR) amplification, cloning, and sequencing of a number of 16S ribosomal RNA (rRNA) genes. The 16S rDNA sequences were clustered into operational taxonomic units (OTUs) and the OTU abundance patterns were obtained for each sample. The observed differences suggest that the chlorine disinfection step markedly affects the bacterial community structure and composition present in GAC water. Members of the *Alphaproteobacteria* and *Betaproteobacteria* were found to be predominant in the GAC water samples after phylogenetic analyses of the OTUs. Following the chlorine disinfection step, numerous changes were observed, including decreased representation of *Proteobacteria* phylotypes. Our results indicate that the use of molecular methods to investigate changes in the abundance of certain bacterial groups following chlorine-based disinfection will aid in further understanding the bacterial ecology of drinking water treatment plants (DWTPs), particularly the disinfection step, as it constitutes the final barrier before drinking water distribution to the consumer's tap.

Keywords Drinking water chlorination · 16S rRNA gene · Phylogeny · Bacterial diversity

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Introduction

The introduction of water treatment for safe use was one of the great achievements of the 20th century. The widespread use of water filtration, followed by disinfection, led to a drastic reduction of infectious disease outbreaks associated with contaminated water [38]. Nonetheless, rapid industrial development, intense agronomic practices, and human demographic changes have resulted in augmented contamination of natural water systems that has challenged the performance of water treatment facilities.

Source water for drinking water production is provided by natural lakes and rivers, manmade reservoirs, and groundwater, depending upon the regionally available freshwater resources. Modern drinking water treatment plants (DWTPs) employ various procedures, including coagulation, flocculation, filtration, and disinfection, depending on source water quality [47]. Previous studies have shown that bacterial diversity in drinking water can be affected by differences in type of source water and treatment processes used in DWTPs [11, 19, 41]. As surface water quality can be affected by a variety of events, such as stormwater runoff or pollution, the treatment processes tailored for groundwater and surface water treatment plants generally differ [22, 47].

In order to reduce organic compound contamination, granular activated carbon (GAC) filtration is often the step preceding the final disinfectant addition step in DWTPs.

GAC filters, a porous media, can accumulate organic nutrients that support bacterial growth and thus metabolism of most remaining contaminants [7, 30, 41, 45]. Bacteria isolated and cultivated from GAC filters have been identified as belonging to a variety of genera, including potential human pathogens [7, 31, 41, 48].

To ensure the microbial quality of drinking water from treatment plant to consumer tap, a final treatment step of disinfection is performed on the GAC effluent water in order to reduce the number of pathogens in the processed water to an acceptable level and to limit microbial growth in the drinking water distribution system (DWDS). In this step, a disinfectant, commonly chlorine or chloramine, is added and subsequently maintained at a minimal residual concentration along the length of the DWDS. Disinfection represents the final step before water entry into the DWDS and thus plays an important role in determining the composition of the bacterial population of finished drinking water [11, 34]. Even in the presence of an extremely potent bactericidal agent such as chlorine, certain bacteria can survive the disinfection procedure. For example, bacterial-colonized GAC particles discharged into the process effluent have been shown to increase resistance to disinfectants and thus lead to the release of bacteria, attached to carbon fines, into the drinking water [8, 26, 29, 41]. Intrinsic resistance to disinfectants commonly used to treat drinking water has also been demonstrated, using cultivation methods, for some members of the *Mycobacterium* and *Bacillus* genera, as well as certain Gram-negative bacterial species [14, 24, 33, 36].

After disinfection treatment, remaining bacteria released into the DWDS may interact with microbial populations present in the water distribution network and be involved in biofilm growth, nitrification, microbial-mediated corrosion, and pathogen persistence [5, 27]. As a consequence, knowledge of the bacterial ecology in the DWTPs is of prime concern for drinking water producers, since the presence of certain bacteria can be a source of water quality problems in the downstream DWDS. Moreover, the appearance of “emerging pathogens” in drinking water has arisen as a new challenge for water and public health authorities. These “emerging pathogens” include species of environmental bacteria that can survive within the DWDS, and comprise a number of opportunistic pathogens, such as *Legionella* sp., *Aeromonas* sp., *Mycobacterium* sp., and *Pseudomonas aeruginosa*, among others [42].

The use of cultivable microbial indicators is required by regulation to assess the biological effectiveness of the treatment processes and quality of finished drinking water. Laboratory experiments, using plate count techniques, have also been used to assess bacterial behavior to disinfectant exposure [14, 24, 33]. Although these cultivation techniques have proven their efficacy in the past, new microbial risk-assessment methods are clearly needed [6, 17], as bacterial culture methods are now known to significantly underestimate the bacterial diversity in drinking water [42]. Molecular methods, based on the amplification and sequencing of the small subunit 16S ribosomal RNA genes (SSU 16S rDNA), have made it possible to study microbial populations independently of cultivation [2]. The use of such 16S rDNA-based approaches conducted on fully functioning surface water DWTPs can provide valuable information on bacterial population changes prior to and after chlorination; for example, a recent 16S rDNA analysis within a groundwater-based DWTP revealed that members of the class *Betaproteobacteria* considerably decreased following the chlorine disinfection treatment [23].

The aim of this study was to assess the bacterial groups present in water after GAC filtration and following chlorination in two DWTPs that use similar treatment processes on different surface water sources (the Marne and Seine rivers) to supply drinking water for the city of Paris, France. For each sample, genomic DNA was extracted and the 16S rRNA genes amplified, followed by DNA sequencing of 439 cloned amplification products. Phylogenetic analyses were performed on the 16S rDNA sequences retrieved from the samples, and taxonomic profiles were obtained for each community. Using this approach, we identified numerous changes in bacterial diversity, including at the levels of both taxa richness and evenness, which occurred after the final disinfection step.

Materials and methods

Water sampling of the two surface water treatment plants

The two DWTPs, located near the towns of Ivry-sur-Seine and Joinville-le-Pont, treat surface water retrieved from the Seine and Marne rivers, respectively, based on biological slow sand filtration [35]. Surface water is first subjected to preozonation, and then filtered through Biolite after the injection of ferric chloride and polyelectrolyte, resulting in contact coagulation. Water is successively filtered through rapid sand filters and biological slow sand filters. After ozonation, followed by GAC filtration, a final disinfection step to produce finished drinking water is carried out by the addition of sodium hypochlorite (free residual chlorine: >0.5 mg/l after 30 min contact time) and followed by the addition of phosphoric acid to inhibit lead pipe corrosion in the DWDS (residual concentration: >1 mg/l).

Granular activated carbon filtered water and finished drinking water from each DWTP were sampled on June 30 and July 1, 2008 from the Joinville-le-Pont and Ivry-sur-Seine sites, respectively, and processed as previously

described [35]. Briefly, water was collected using sterile 1-l bottles containing 20 mg sodium thiosulfate to inactivate residual chlorine and ozone. Samples were transported on ice and processed within 4 h after collection. Microorganisms were harvested by filtering 12 l water through a 0.2-μm-pore-size nylon filter (47 mm diameter, Millipore, Molsheim, France) and the filters were stored at −70°C until use.

Nucleic acids extraction and purification

Nucleic acids were extracted from the frozen filters as previously described [35]. As nucleic acids extracted from the GAC samples were found to contain significant amounts of RNA, known to cause problems in PCR reactions [44], the extracted nucleic acids from all samples were treated with 50 U RNase I (Fermentas, Saint-Remy-lès-Chevreuse, France) for 10 min at 37°C. The nucleic acids were then purified by chloroform/isoamyl alcohol extraction, precipitated with ethanol, and resuspended in 0.1× TE buffer, as previously described [35]. The purified nucleic acids were visualized by electrophoresis through a 0.8% agarose gel in TAE buffer (20 mM Tris–acetate pH 8, 5 mM Na-EDTA) and stored at −20°C until use.

Small subunit rRNA gene library construction

The 16S rDNA sequences were amplified from DNA samples using the primers 517F (5′-GCCAGCAGC CGCGGTAA-3′) and 1407R (5′-GACGGGCGGTGTGT RC-3′) [50]. The PCR reactions (five per sample, to avoid single-tube amplification bias) were carried out in 50 μl total final reaction volumes using 10 ng DNA template, 0.25 μM phosphorylated primers (Sigma–Aldrich, Lyon, France), 0.2 mM dNTP mix (Fermentas, Saint Remy Les Chevreuses, France), 1× Phusion HF buffer (Ozyme, Saint-Quentin-en-Yvelines, France) with 1.5 mM $MgCl_2$ and 0.5 U Phusion DNA polymerase (Ozyme, Saint-Quentin-en-Yvelines, France). The conditions of amplification were: 2 min at 98°C followed by 25 cycles for 20 s at 98°C, 30 s at 54°C, 20 s at 72°C, and a final elongation step for 5 min at 72°C. The amplified fragments were purified using the Nucleospin Extract II kit (Macherey–Nagel, Hoerdt, France) after electrophoresis through a 0.8% agarose gel in TAE buffer. Then, the purified DNA fragments were cloned into the pSmartLCKan vector (Lucigen, Middleton, USA) as recommended by the manufacturer. Plasmids containing 16S rDNA-sized inserts were isolated using the Nucleospin Multi-96 Plus Plasmid kit (Macherey–Nagel, Hoerdt, France) and sequenced by Cogenics-GENOME Express (Grenoble, France) using vector primers.

16S rDNA sequence and phylogenetic analyses

After sequencing of selected cloned inserts, chimeric 16S rDNA sequences were removed after examination using the Bellerophon [18] and Pintail [3] programs. The remaining sequences retrieved from all samples were aligned using the NAST program [10], manually refined with Bioedit version 7.0.0 [16], and then a corrected distance matrix (F84 model) was generated using the DNADIST version 3.5c program from the PHYLIP package [13] implemented in Bioedit. The generated distance matrix was used as an input file for DOTUR version 1.3 [37]. The DOTUR program was used to group sequences into defined single operational taxonomic units (OTUs) using a ≥97% sequence similarity threshold [40], and to calculate total OTU richness (Chao1 estimates). Representatives of each OTU were selected and subjected to a phylogenetic analysis. In total, 168 16S rDNA sequences were used as a BLAST query [1] against 16S rDNA sequences present in the GenBank and Ribosomal Database Project II databases [4, 9]. The closest matching sequences were retrieved from the databases and aligned with the cloned sequences using NAST, and then manually refined with Bioedit. The bootstrapped (100 replicates) phylogenetic trees were generated with MEGA version 4 [43] using the neighbor-joining and maximum-likelihood methods. The 16S rDNA sequences determined in this study have been deposited in the GenBank database under accession numbers GQ452964 to GQ453402.

Results

OTU abundance patterns

Water samples were collected from the GAC filter effluent and after the final chlorine disinfection step of the treatment process from the two DWTPs. The bacteriological and chemical analyses of the water sources (Seine or Marne rivers) used by the DWTPs, and the GAC effluents plus finished drinking water samples, are presented in Table 1. Although the samples collected at the two water sources did not display major differences, the results did reveal higher total coliforms and *Escherichia coli* numbers in the Seine river and higher levels of NH_4 in the Marne river (Table 1).

A 16S rDNA library was generated for each sample and 439 total sequences were analyzed after removal of chimeric sequences. The 16S rDNA libraries were designated I-GAC and I-DW, for the Ivry-sur-Seine GAC and finished drinking water samples, respectively, and J-GAC and J-DW, corresponding to the Joinville-le-Pont GAC and finished drinking water samples, respectively. In order to

Table 1 Summary of water quality at the time of sampling

Parameter measured	Site					
	Ivry-sur-Seine			Joinville-le-Pont		
	Seine river	GAC	DW	Marne river	GAC	DW
Temperature (°C)	24.0	26.9	23.3	27.4	24.9	24.8
pH	8.15	7.70	7.47	8.20	7.95	7.90
Turbidity (NFU)[a]	7.69	0.042	0.046	8.30	0.035	0.028
TOC (mg/l)[b]	2.52	1.46	1.57	1.96	1.19	1.39
Free chlorine (mg/l)	–	–	0.54	–	–	0.72
Total chlorine (mg/l)	–	–	0.62	–	–	0.79
NH_4 (mg/l)	0.03	<0.01	<0.01	0.09	<0.01	<0.01
NO_2 (mg/l)	0.070	<0.005	<0.005	0.060	<0.005	<0.005
E. coli (/100 ml)[c]	3,000	0	0	1,180	0	0
Total coliforms (/100 ml)[c]	8,000	0	0	2,334	0	0

measure the potential bacterial richness and abundance within each sampled library, the 16S rDNA sequences with ≥97% sequence similarity were clustered into operational taxonomic units (OTUs) and the OTU abundance pattern was determined by ranking and plotting the relative abundance data for each sample (Fig. 1). The results yielded 28, 33, 73, and 55 distinct OTUs in the J-DW, I-DW, J-GAC, and I-GAC samples, respectively. The finished drinking water samples were found to contain several abundant (each accounting for ≥10% of the population) OTUs representing 22%, 18.9%, and 12.1% of the total 16S rDNA sequences in the J-DW sample and 23.8%, 13.3%, and 12.4% of the total 16S rDNA sequences in the I-DW sample (Fig. 1). In contrast, less than 10% of the total sequences could be classed as "abundant OTUs" in the GAC effluent samples, suggesting a more uniform population structure. In support of this, we found that the frequency of single-represented sequenced clones accounted for 10.6% and 16.2% of the 16S rDNA sequences in the J-DW and I-DW samples, respectively, versus 53.9% and 79.5% of the 16S rDNA sequences in the I-GAC and J-GAC samples, respectively (Fig. 1). Moreover, species diversity based on Chao1 estimates indicate a total OTU richness projected to be 43 (J-DW) and 62 (I-DW) for the finished drinking water samples, and 238 (J-GAC) and 125 (I-GAC) total OTUs in the GAC effluent samples.

Phylogenetic analyses

Representatives of each OTU were subjected to a phylogenetic analyses (Figs. 2, 3, 4). In the GAC samples, a variety of the OTUs were found to belong to the *Proteobacteria* phylum (Figs. 2, 3). The *Alphaproteobacteria*-affiliated OTUs were found to be clustered into 21 and 29 OTUs in the I-GAC and J-GAC samples, respectively, whereas only 2 (J-DW) and 5 (I-DW) *Alphaproteobacteria*-affiliated OTUs were discerned in the finished drinking water samples (Fig. 2). The assigned *Alphaproteobacteria* sequences were found to be affiliated to a variety of taxa, including members of the *Sphingopyxis*, *Brevundimonas*, *Hyphomicrobium*, *Methylocystis*, *Bradyrhizobium*, *Rickettsia*, and *Acidisphaera* genera. Within the class *Betaproteobacteria*, 17 distinct OTUs were identified in the GAC samples. Among these were members belonging to the *Methylibium*, *Polaromonas*, *Comamonas*, *Herminiimonas*, *Polynucleobacter*, and *Methylophilus* genera (Fig. 3a), and including the J-GAC-19 and J-GAC-17 OTUs found to be closely related to 16S rDNA sequences (sequences with a BAC prefix in Fig. 3a) retrieved from cultivated bacteria previously isolated from GAC filters [31]. OTUs affiliated to the *Gammaproteobacteria* were represented only by members belonging to the genus *Legionella* and were only observed in the GAC samples (Fig. 3b). The phylogenetic analyses also revealed the presence of OTUs belonging to the class *Deltaproteobacteria*, some of which were found to be related to members of the genus *Bdellovibrio* (Fig. 3c).

Phylogenetic analyses of the OTUs retrieved from the chlorinated finished drinking water samples showed that members belonging to nonproteobacterial groups were numerous in both finished drinking water samples (Fig. 4). The most frequently observed OTUs in the I-DW and J-DW samples were found to be closely related to those (sequences with an HOCl or Ivry prefix in Fig. 4) previously observed in other drinking water samples [35, 49]. More precisely, the J-DW-87 (representing 25 clones in the J-DW and I-DW samples) and J-DW-46 (representing 29 and 6 clones in the J-DW and I-DW samples, respectively) OTUs were affiliated to currently unclassified bacteria (Fig. 4). Within the *Bacteroidetes*, all OTUs were capable of being placed into the orders *Sphingobacteriales* and

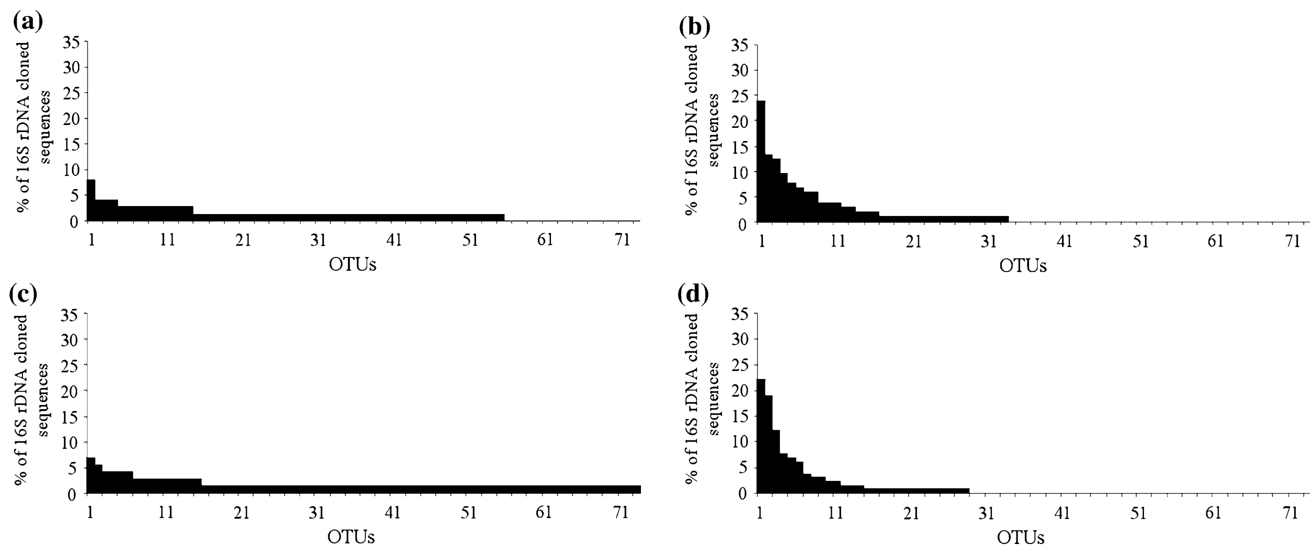

Fig. 1 Rank abundance plots of OTUs from each sampled library: **a** GAC and **b** finished drinking samples from Ivry-sur-Seine; **c** GAC and **d** finished drinking water samples from Joinville-le-Pont

Flavobacteria and some of these were found to be closely related to 16S rDNA sequences affiliated to the genera *Terrimonas* and *Chryseobacterium* (Fig. 4). The I-DW-24 and I-DW-11 OTUs, represented by 14 and 13 clones, respectively, were found to be highly related to *Mycobacterium salmoniphylum* (99.8% sequence similarity) and *Mycobacterium llatzerense* (100% sequence similarity) (Fig. 4). Eight OTUs retrieved from the finished drinking water sample from the Joinville-le-Pont DWTP were identified as belonging to the *Planctomycetes* phylum (Fig. 4). The remaining sequences from the samples were scattered over a wide taxonomic distribution, including members belonging to the phyla, *Acidobacteria*, *Chlamydiae*, *Verrucomicrobia*, *Nitrospira*, *Firmicutes*, and *Cyanobacteria*.

Taxonomic composition

The percentage of 16S rDNA sequences affiliated to the major bacterial taxonomic groups present in each sample is shown in Fig. 5. The results revealed that the total sequenced clones affiliated to the *Proteobacteria* phylum accounted for 15.5% and 18.4% of the 16S rDNA sequences in the J-DW and I-DW samples, respectively, with a significantly higher proportion (81.5% for J-GAC and 71.1% for I-GAC) observed in the GAC samples (Fig. 5). At least 50% of the clones retrieved from the I-GAC and J-GAC samples were assigned to the classes *Betaproteobacteria* and *Alphaproteobacteria*, whereas in the finished drinking water samples, only 16.9% (I-DW) and 2.2% (J-DW) of the clones were affiliated to these proteobacterial classes (Fig. 5).

Among the other *Eubacterium* phyla, both the J-DW and I-DW samples contained a large proportion (51.5% and 35.9%, respectively) of 16S rDNA clones belonging to a bacterial group composed of currently unclassified members. Sequences affiliated to the phylum *Bacteroidetes* were observed at higher frequencies in the finished drinking water samples than in the GAC water samples from both sampled sites (Fig. 5). We observed an increased abundance of sequences affiliated to the phylum *Actinobacteria* between the I-GAC (1.3%) and I-DW (23.7%) samples, yet no member of this phylum was observed in the Joinville-le-Pont samples. In the J-DW sample, members of the *Planctomycetes* were found to account for 21.3% of the observed bacterial population (Fig. 5).

Discussion

The aim of the present study was to investigate bacterial community changes in processed water after the disinfection step in two surface water treatment plants using a 16S rDNA-based approach to overcome cultivation-based limitations. We examined the bacterial community composition of treated water samples taken at the end of the GAC filtration step and after chlorine-based disinfection from the Ivry-sur-Seine and Joinville-le-Pont DWTPs that use similar treatment processes but different raw water sources (Marne and Seine rivers).

Microbial cells were collected from water samples by filtration, DNA was extracted, and the 16S rRNA genes amplified by PCR. After clone library construction, sequenced 16S rRNA genes were grouped into OTUs using

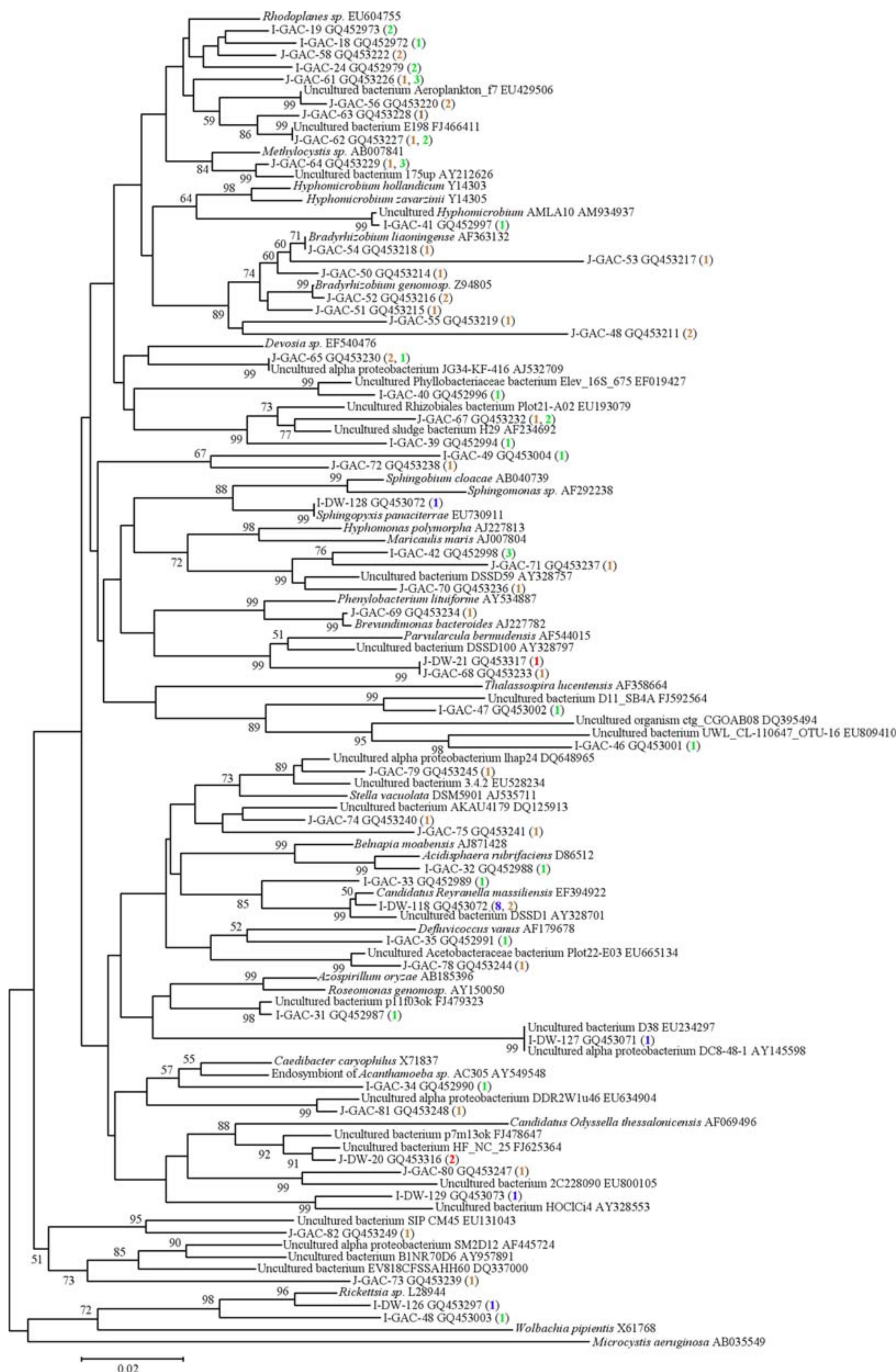

Fig. 2 Phylogenetic tree of the OTUs belonging to the class *Alphaproteobacteria*. Bootstrap values greater than or equal to 50% are indicated at the nodes. The *scale bar* represents the number of substitutions per unit branch length. The *numbers in parentheses* indicate the occurrence of a specific OTU in the different 16S rDNA sampled libraries as follows: *green*, I-GAC sample; *blue*, I-DW sample; *brown*, J-GAC sample; and *red*, J-DW sample. The tree was rooted using *Microcystis aeruginosa* as an outgroup

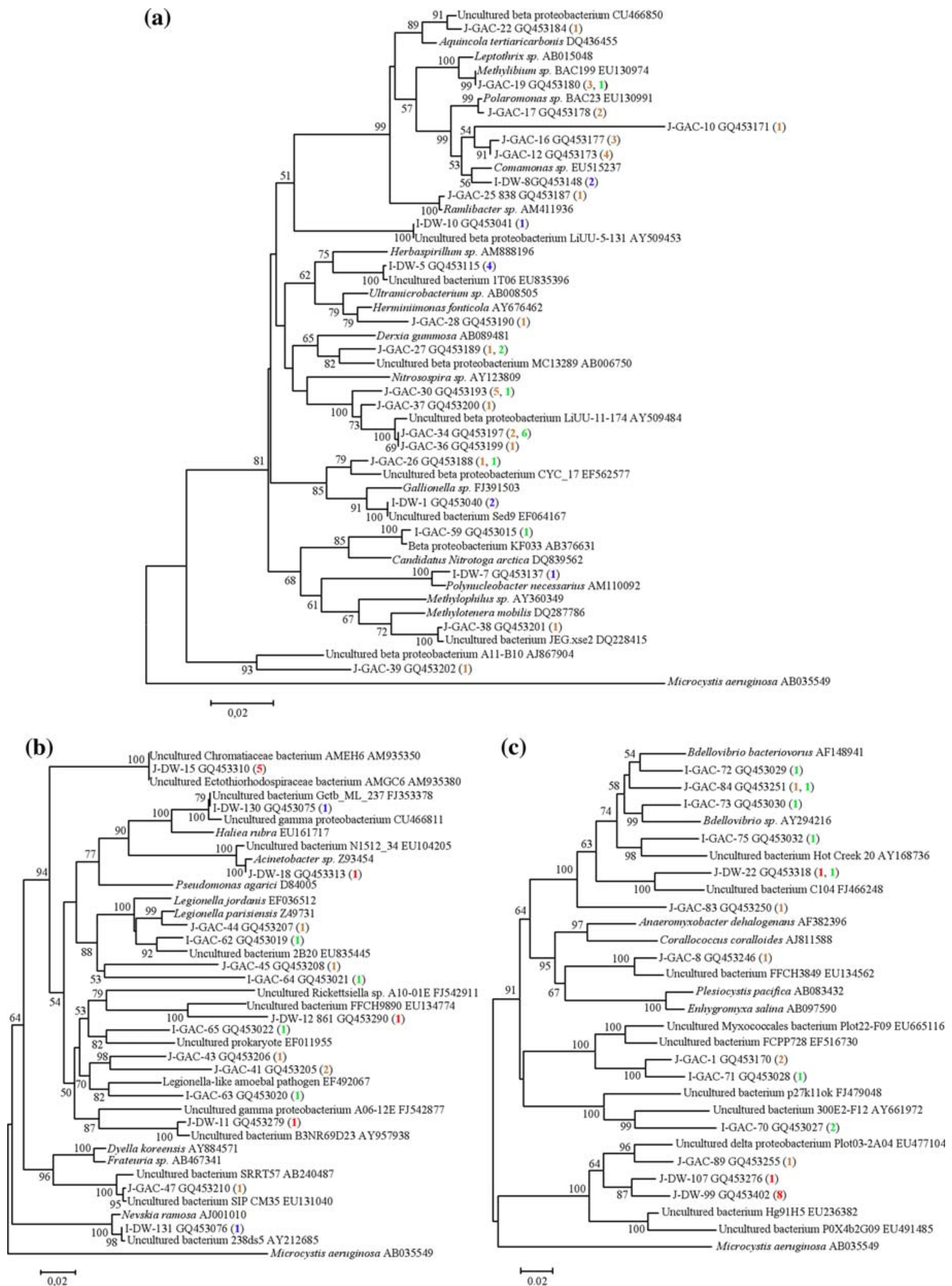

Fig. 3 Phylogenetic trees of the OTUs belonging to the classes **a** *Betaproteobacteria*, **b** *Gammaproteobacteria*, and **c** *Deltaproteobacteria*. Bootstrap values greater than or equal to 50% are indicated at the nodes. The *scale bar* represents the number of substitutions per unit branch length. The *numbers in parentheses* indicate the occurrence of a specific OTU in the different 16S rDNA sampled libraries as indicated in the legend to Fig. 3. The trees were rooted using *Microcystis aeruginosa* as an outgroup

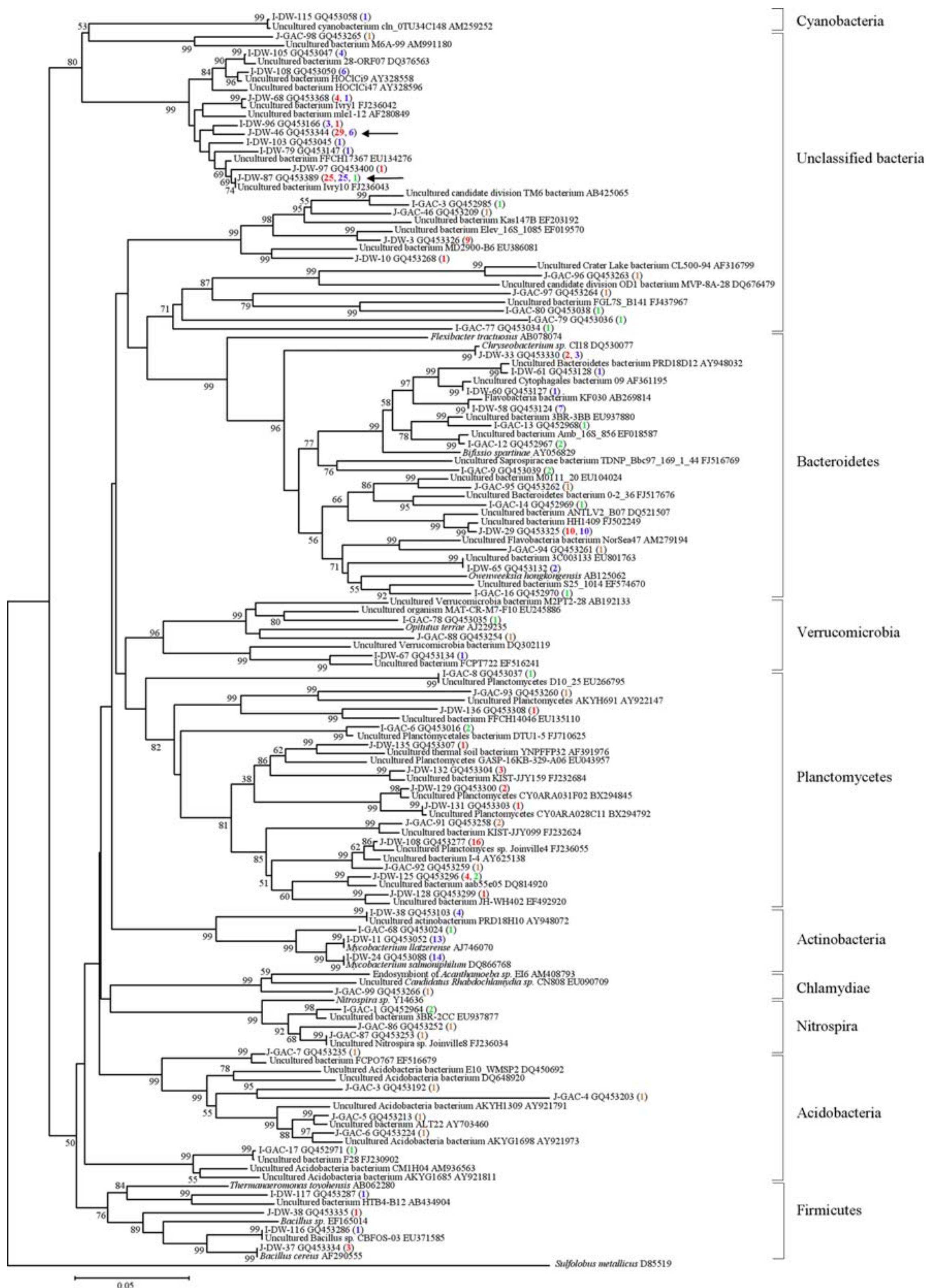

Fig. 4 Phylogenetic analysis of the OTUs belonging to the domain *Eubacteria*. Bootstrap values greater than or equal to 50% are indicated at the nodes. The *scale bar* represents the number of substitutions per unit branch length. The *numbers in parentheses* indicate the occurrence of a specific OTU in the different 16S rDNA sampled libraries as indicated in the legend to Fig. 3. *Arrows* indicate the most abundant OTUs present in the I-DW and J-DW samples. The tree was rooted using *Sulfolobus metallicus* as an outgroup

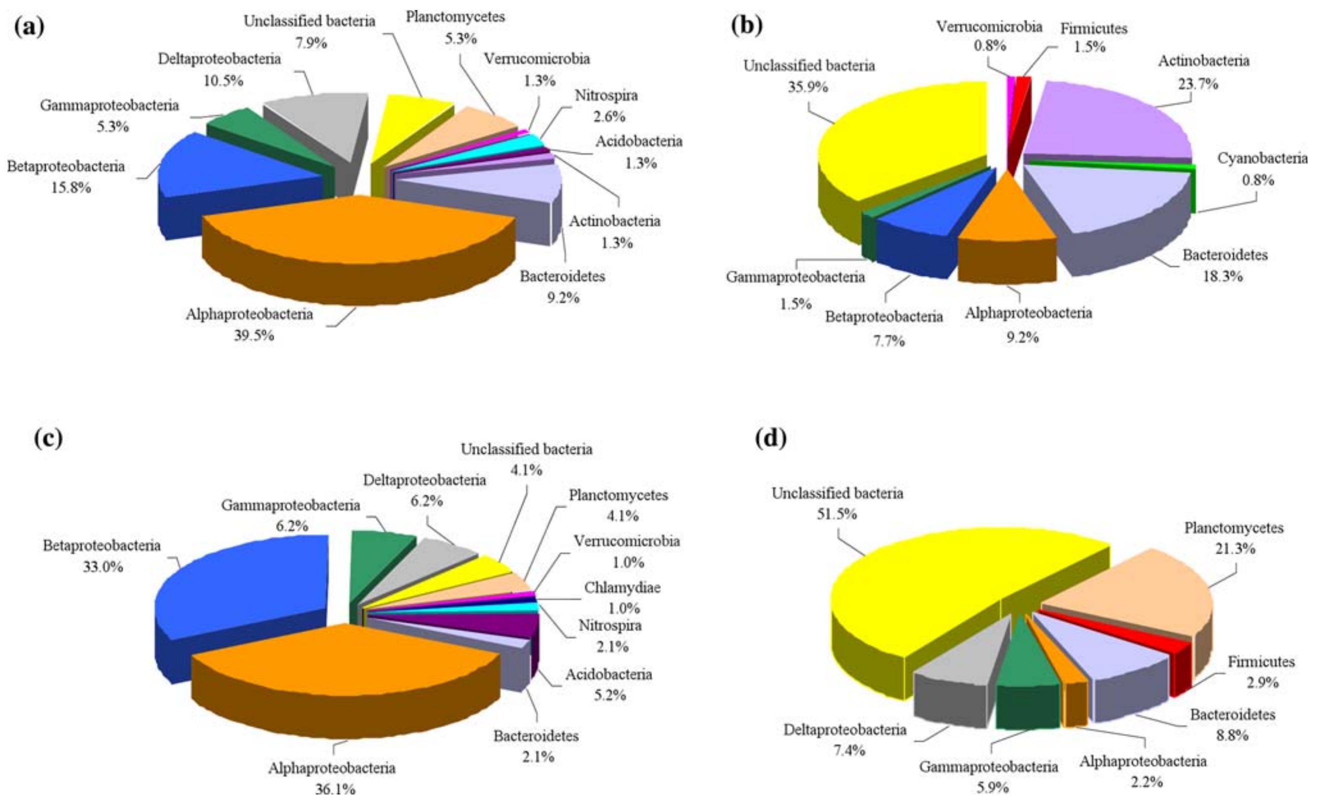

Fig. 5 Taxonomic abundance of 16S rDNA sequences from each sampled library: **a** GAC and **b** finished drinking water samples from Ivry-sur-Seine; **c** GAC and **d** finished drinking water samples from Joinville-le-Pont

a $\geq$97% sequence similarity threshold. The OTU richness identified in the finished drinking water libraries [28 (J-DW) and 33 (I-DW) OTUs] was found to be less than that observed in the GAC water samples [55 (I-GAC) and 73 (J-GAC) OTUs]. The Chao1 calculations indicate an overall estimated total OTU richness of 43 (J-DW) and 62 (I-DW) OTUs in the finished drinking water samples, whereas 125 (I-GAC) and 238 (J-GAC) OTUs were estimated to be present in GAC water samples. These results, not surprisingly, show a decrease in bacterial richness present in the treated water following the disinfection step, a result consistent with previous studies [23, 34] and in accordance with the known biocide effects of adding uncombined chlorine in the form of hypochlorous acid (HOCl). Our results also show the presence of several dominant OTUs in the finished drinking water samples from both the Ivry-sur-Seine and Joinville-le-Pont samples, whereas this dominance was not observed in the corresponding GAC effluent samples. An explanation for these bacterial evenness changes following the disinfection treatment is likely attributable to a differential level of sensitivity of bacterial taxa present in the GAC water to the chlorine disinfectant [24, 32].

At the end of GAC filtration, the bacterial population was found to be dominated by a variety of low abundance OTUs, mainly affiliated to the *Alphaproteobacteria* and *Betaproteobacteria* classes in both the I-GAC (71.1%) and J-GAC (81.5%) samples (Figs. 2, 3a). These results are consistent with those obtained using cultivation methods on GAC effluent samples, as the colonies identified as belonging to the genus *Pseudomonas* are presently classified within the *Proteobacteria* phylum, and mainly affiliated to the *Alphaproteobacteria* and *Betaproteobacteria* classes [21, 34, 41]. By identifying the predominant cultivable bacteria within GAC filters from different full-scale DWTPs, Magic-Knezev et al. (2009) also found that most bacteria belonged to the *Alphaproteobacteria* and *Betaproteobacteria* classes, suggesting that these types of bacteria, or their nucleic acids, may persist up until this step in the DWTPs.

Following the chlorine disinfection step, reduced numbers of *Proteobacteria*-affiliated clones, particularly marked for the *Alphaproteobacteria* and *Betaproteobacteria* classes, were encountered in both the J-DW (15.5%) and I-DW (18.4%) samples (Fig. 5). It has been previously noted that members of the *Betaproteobacteria* can be particularly sensitive to chlorine disinfection [23, 49]. Members of the *Proteobacteria* have, in contrast, been previously observed to be abundant in some samples of finished drinking water [35] or in planktonic bacterial

populations of downstream chlorinated drinking water within an urban DWDS [49]. These differences can be accounted for by differences in the location of different drinking water networks, source water quality [11] or by physicochemical effects, such as water temperature, on the efficiency of chlorine treatment [20, 24]. In addition, the presence of microbial biofilms, including members of the genus *Sphingomonas* [39], on the pipe surfaces within a DWDS, and subsequent release of biofilm-attached bacteria into the bulk water phase, may also affect the planktonic bacterial diversity observed in drinking water [28].

The 16S rDNA sequences affiliated to an unclassified bacterial group, as well as members of the *Bacteroidetes*, *Planctomycetes*, and *Actinobacteria* phyla, constitute the most abundant bacterial lineages observed in the finished drinking water samples (Fig. 5). Phylogenetic analyses of clones examined in the I-DW and J-DW samples revealed that the highest abundance OTUs, affiliated to the unclassified bacteria observed in the I-DW and J-DW samples, were identical (Fig. 4) and closely related to those observed in previous studies [35, 49], suggesting that these bacteria may form part of the microbiota present in chlorinated drinking water. The *Bacteroidetes*, identified in higher abundance after the disinfection step in both the I-DW and J-DW samples, were found to be represented by members of the *Sphingobacteriales* and *Flavobacteria* orders (Fig. 4). Members of the *Bacteroidetes* (previously known as *Cytophaga–Flavobacterium–Bacteroides*) are frequently isolated in aquatic environments [52]. *Flavobacterium* species have been reported to display a high degree of resistance to chlorine treatment and be readily isolated from disinfected drinking water [41, 51]. Members belonging to the *Planctomycetes* phylum were found to represent 21.3% of the total 16S rDNA sequences retrieved in the J-DW sample (Fig. 5), a result in accordance with previous studies, as members of the phylum *Planctomycetes* have been consistently identified in chlorinated drinking water [11, 23, 35]. Members of the *Actinobacteria* were found in the I-GAC (1.3%) and I-DW (23.7%) samples (Fig. 5). The phylogenetic analyses indicate that the OTUs affiliated to the genus *Mycobacterium* within the *Actinobacteria* in the Ivry-sur-Seine finished water sample were highly related (>99%) to *Mycobacterium salmoniphylum* and *Mycobacterium llatzerense* (Fig. 4). Whereas *Mycobacterium salmoniphylum* has been considered as a *Mycobacterium chelonae*-like organism isolated from salmonid fishes, *Mycobacterium llatzerense* was isolated from hemodialysis water [15, 46]. The significance of their presence is not currently discernible. The presence of Mycobacteria in drinking water, and specifically in the DWTP of Ivry-sur-Seine, has previously been reported [12, 25]. Moreover, members of the genus *Mycobacterium* have been found to be resistant to chlorine treatment, and some species (e.g., *Mycobacterium tuberculosis*) are known to be pathogenic for humans [24, 25]. No 16S rDNA sequences capable of being affiliated to the genus *Mycobacterium* were identified in the Joinville-le-Pont DWTP samples, a result possibly due to quantitative differences in their presence in the two source waters used and/or slight functional differences between the two DWTPs [12]. It should be noted here that it is not possible to determine if the bacteria that we identified represent dead or live cells. Although a 16S rDNA-based analysis allows bacterial identification in the absence of cultivation-based bias, the significance of the bacterial groups detected in this study clearly requires much further research.

The results reported here support the notion that chlorination plays an important role in the bacterial populations of finished drinking water released into the DWDS [11, 34]. The data presented in this study show that the disinfection step, carried out by chlorine addition in the DWTPs, can markedly affect the bacterial diversity in finished drinking water. Our results also indicate that certain bacterial groups are particularly affected by the chlorine-based disinfection treatment performed in the two DWTPs, suggesting a differential level of sensitivity to the disinfection treatment. Further research is clearly needed to fully elucidate the bacterial ecology of DWTPs, particularly the disinfection step, as it constitutes the final barrier before drinking water distribution to the consumer's tap.

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