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Study on Microbial Community Structures in Drinking Water Sludge by PCR-DGGE

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In order to offer useful information for harmless disposal of drinking water sludge, the bacterial community structures of sludge produced in two different drinking water plants were initially studied by polymerase chain reaction–denaturing gradient gel electrophoresis (PCR–DGGE) technique. The result of sequencing of DGGE band analysis showed that the microbial community structure of drinking water sludge was complex, various types and a large number of microbes lived in drinking water sludge according to the Shannon-Wiener index of diversity (H) and the specific richness (R). Nine phyla obtained by the similarity analysis of 27 strong bands selected from the DGGE profiles sludge samples as follows: *Proteobacteria, Acidobacteria, Fibrobacteres, Chloroflexi, Bacteroidetes, Firmicutes, Cyanobacteria, Verrucomicrobia* and *Sheathe bacteria*. Among them, *Proteobacteria* contained two classes (*Gamma-proteobacteria* and *Beta-proteobacteria*) and then three genera (*Rhodocyclus, Proteobacterium* and *Methylothermus*) were the most common species. *Chloroflexi* including three classes (*Chloroflexi*, *Caldilineae* and *Anaerolineae*) and *Bacteroidetes* (*Bacteroidetes* and *Flavobacteria*) were also usual populations. Most of species, with high organic materials degradation activity, were heterotrophic bacteria due to a large number of organic materials contained in drinking water sludge. The present study also demonstrated the comparison of microbial community structure between drinking water sludge and wastewater sludge, *Proteobacteria*, *Bacteroidete*s and *Chloroflexi* were considered as the most common dominant species on phylum level, Differences such as the number of *Rhodocyclu* in drinking water sludge or *Micrococcus* in wastewater sludge were obviously shown due to the different treatment process and the inlet water quality.

Key words: Drinking Water, Sludge, PCR-DGGE, Microbial Community Structure, Bacteria.

Drinking-water treatment sludge is a by-product generated by coagulation with a hydrolysing metal salt such as aluminium sulfate ('alum') or ferric chloride ('ferric') which are used as coagulants to remove colour, turbidity and humic substances (Verrelli *et al*., 2009; Razali *et al*., 2007). As a result of this operation, several million tons of clarifier sludge contained suspended solids, colloidal matter and color-causing organics in natural water are produced yearly[1] (Petruzelli *et al*., 2000) and some organohalogen contaminants, pathogens (bacteria, viruses and protists) and concentrated metals, e.g., aluminum and iron may also live in it (Hall *et al*., 1989; Rivera *et al*., 1997; Wu¨ rzer *et al*., 1995; Bourgeois *et al*., 2004).

Concern has gradually risen owing to the urgent demands to reduce waste disposal costs and environmental impacts. The sludge treatment process in place at drinking water treatment plant (DWTP) includes the following stages: sludge gathering and storage, pumping to thickening area, thickening, storage of thickened sludge, pumping to dehydration area, dehydration, atomization and final storage (Wang *et al*., 2005). Determination of

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microbial community structure is important on drinking water sludge treatment, but the shortage of information is due, in part, to the lack of enough attention for analyzing microbial community structure and diversities in environmental samples. Recently, the PCR- DGGE approach is widely used by most researchers in environment study, such as fermentation, soil or sea (Edenborn *et al*., 2007; Hamasaki *et al*., 2007). It could offer a lot of available information for the microbial community structure of many environmental samples and provide a valuable basis for further study on bacteria characteristics.

Culture techniques are depended on by the conventional analysis of microbial communities (Yoshie *et al*., 2001). However, culture-dependent approaches which need a waste of time and fussy operation are biased by only a fraction of selected species which don't demonstrate the real dominance structure. Different culture-independent methods to fingerprinting, such as denaturing gradient gel electrophoresis (DGGE; Kocherginskaya *et al*., 2001; Smit *et al*., 1997), real-time PCR (Du *et al*., 2006), fluorescent in situ hybridization (FISH) (Dong *et al*., 2010), amplified ribosomal DNA restriction analysis (ARDRA) and clone libraries, terminal restriction fragment length polymorphism (T-RFLP) (Gong *et al*. 2002; Eriksson *et al*., 2003) are widely used to characterize the microbial communities and to identify individual members based on V3-16SrDNA for ecological studies. The use of DGGE followed by PCR-amplification is to assess the diversity of microbial structure and determine the phylogeny of community members by analyzing the sequence of DNA fragments after they are showed from the gel in which bands corresponding to each species of microbe have been separated by DGGE.

So far, to our knowledge, researches on microbial community structure of activated sludge in wastewater treatment plant are numerous. However, there have been few studies on that of drinking water sludge. In this study, the microbial community structure of the drinking water sludge was systematically researched by PCR-DGGE technology and the characteristics of the selected bacteria were mentioned. In addition, the comparison existed between activated sludge in wastewater and drinking-water treatment processes in terms of microbial community structure was demonstrated in order to provide useful information for harmless disposal of sludge produced in drinking water process.

MATERIALS AND METHODS

DNA extraction and purification

The sludge samples collected respectively from the flow tank of two drinking water plants in two different cities were all centrifuged for 10min at 12000r/min within 12hrs and then the total DNA was extracted respectively using Soil DNA Fast Extraction Kit (Spin-column) according to the manufacturer's instructions (Bio Teke, China).

PCR amplification and product detection

Bacterial specific universal primers, 341F with GC-clamp and 534R, were used to amplify the V3 region of 16SrDNA gene, (Muyzer *et al*., 1993; Xing *et al*., 2006). PCR amplification was performed in a 50ul reaction mixtures and carried out in an authorized mastercycler (Eppendorf, Germany) according to standard protocols (Choi *et al*., 2007).

DGGE of composite PCR products (20ul) were applied directly onto a polyacrylamide gel (Nakasaki *et al*., 2009; Nadarajah *et al*., 2007) and the electrophoresis was performed at 60°C and 150 V for 280min (Liu *et al*., 2008).Images were captured using Quantity One 4.3.0 gel analysis software (Bio-Rad, USA) to evaluate the diversity indices of the microbial community calculated from the DGGE band profiles. The Shannon-Wiener index of diversity (H) (Shannon *et al*., 1963), the the equitability index (E) (Pielou, 1975) and the Dice index (Cs) (Dice, 1945) were used to present the diversity of the bacterial community.

Sequence alignment and phylogenic tree

PCR products were cloned according to the manufacturer's instruction and then sequenced by Sangon Biotech (Shanghai) Co. Ltd. Clone sequences recovered from excised bands were manually compared to the GenBank database to identify the most similar 16SrDNA sequences selected with more than 93% homology taxonomically by using the alignment basic local search tool (BLAST) (Regina *et al*., 2003) and classified them by Ribosomal Database Project (RDP) (Table 3) in order to investigate the phylogenetic identities. The nucleotide sequences were aligned with the CLUSTAL-X program

(Thompson *et al*., 1997) and the phylogenetic trees were constructed by the neighbor-joining method (Saitou *et al*.,1987) using MEGA 4.

Nucleotide sequence accession numbers

The sequences obtained in this study are available in the GenBank database under accession numbers: JN936813-JN936838 and JQ012796.

RESULTS AND DISCUSSION

Analysis of DGGE profiles

A total of 27 strong DGGE bands were isolated from the different positions of the gel, some minor bands were not been identified because they could not be excised from the gels due to their low intensities, so bands with a relative intensity of less than 0.58% of the sum of all band intensities were discarded. As shown in Table 1, the indices of H, E and R, reflecting the structural diversity of the bacterial community (Gafan *et al*., 2005), were calculated on the basis of the number and relative intensities of bands in the gel. The Shannon-Wiener index of diversity (H) ranged from 3.372 to 3.525 and the specific richness (R) (from 34 to 39) were used to calculate the diversity of bacterial communities, they were demonstrated that the bacterial community structures of sludge samples were complex and the species were multitudinous. Equitability index can range from near 0, indicating pronounced dominance, to near 1, indicating complete evenness, i.e. (Pielou, 1975). The analysis of equitability index (E) ranged from 0.956 to 0.9612 was showed an almost consistent distribution of taxa between sludge samples. Higher H and E values were registered in sample C, indicating relative abundances and higher number of species in stale sludge sample of Plant A, compared to the fresh sludge sample.

The similarity (Dice coefficient, 84.23%) between fresh sludge sample of Plant A (Lane A_1) and that of Plant B (Lane B) was very high due to many common species, the same coagulant and technology in treatment process between the two plants might be the main reason for the high similarity of bacterial community structures. The profile of the fresh sludge sample of Plant B (Lane B) had about 78.34% community similarity to the stale sludge sample of Plant B (Lane C), this phenomenon maybe results from the effect of the standing time.

Although total numerical analysis of the DGGE patterns of sludge microbial communities in drinking water showed a few changes, the selected dominant bands identified from DGGE profile were different due to the difference of the water quality. As shown in Table 2 and Figure 1, bands 3, 13, 14, 16 and 26 in the profile of Lane A_1 were found to have strong intensity whose OD value were 4.628, 3.046, 2.976, 2.575, 2.512 respectively, whereas those in Lane B were very faint (1.026, 0.987, 0.884, 1.757, 1.011 relatively) and bands 4, 9, 12, 17 and 18 were abundant in Lane B with OD value 3.994, 3.467, 3.112, 2.504 and 2.911 respectively but lower in Lane A_1. Lane B had stronger intensities bands such as bands 15, 17, 18, 19, 20 and 23 (OD value 2.513, 2.504, 2.911, 2.566, 2.499 and 3.763 respectively) than Lane C, although the intensities of bands 2, 8, 10, 11, 13 and 14 with OD value 5.297, 3.253, 3.704, 2.762, 3.046, 2.976 respectively were much higher in Lane C compared to Lane B in which each relative band was low to 1.898. The results were indicated that the fresh sludge samples of different plants not only contained many common bacterial groups but also a few particular species in which existed respectively, the differences in source water quality such as the concentrations and types of DOC between the two plants might be the main reason for the small differences of bacterial community structures except the same treatment process and added coagulant. As the residence time increased, the species of the sludge bacterial community in the same plant were not much alteration but the quantities were subject to change, some specific species were affected as a result of the dissolved oxygen and the organic materials of the sludge gradually reduced.

Table 1. Structural biodiversity (H), specific richness (R) and equitability index (E) calculated from the digitized DGGE patterns

	Lane A_1	Lane B	Lane A_2	Lane C
H	3.496	3.375	3.501	3.372
E	0.961	0.957	0.962	0.956
R	38	34	39	34

Lane A_1, A_2: fresh sludge sample of Plant A; Lane B: fresh sludge sample of Plant B; Lane C: stale sludge sample of Plant B

Table 2. Similarity of sequences of selected DGGE bands, as determined by BLAST nucleotide search

Band no.	Closely related sequences	Accession no.	Similarity	Relative OD value		
				Lane A_1	Lane B	Lane C
1	*Bacteroidetes* bacterium (EF636477)	JN936813	97	0.667	3.598	3.330
2	*Verrucomicrobia* bacterium (CU920931)	JN936814	95	0.564	1.898	5.297
3	*Acidobacteria* bacterium (AM935817)	JN936815	95	4.628	1.026	3.792
4	*Verrucomicrobia* bacterium (EU299285)	JN936816	93	0.687	3.994	3.012
5	*Rhodocyclaceae* bacterium (HQ003468)	JN936817	97	2.167	0.963	2.990
6	*Rhodocyclaceae* bacterium (GU472572)	JN936818	97	1.723	1.998	2.077
7	Uncultured *Fibrobacteres* bacterium (HQ386512)	JN936819	99	1.679	0.602	1.637
8	*Chloroflexi* bacterium (FJ916298)	JQ012796	99	2.236	1.121	3.673
9	*Escherichia coli* (GU415870)	JN936820	95	1.726	3.467	3.253
10	Uncultured bacterium (AB205886)	JN936821	98	0.569	0.664	3.704
11	Uncultured *cyanobacterium* (FN860124)	JN936822	98	2.032	0.896	2.762
12	*Fibrobacteres* bacterium (DQ501290)	JN936823	99	0.685	3.112	3.294
13	*Chloroflexi* bacterium (FJ916310)	JN936824	100	3.046	0.987	2.745
14	Uncultured *Chloroflexi* bacterium (JN379247)	JN936825	98	2.976	0.884	2.258
15	*Bacteroidetes* bacterium (EU283360)	JN936826	96	2.128	2.513	0.898
16	*Methylothermus sp.* (FM176282)	JN936827	96	2.575	1.757	1.738
17	*Eubacterium* (AF495405)	JN936828	98	1.012	2.504	0.967
18	*Anaerolineae* bacterium (EF491509)	JN936829	94	1.121	2.911	0.983
19	Uncultured *Flavobacteriaceae* bacterium (FJ764434)	JN936830	93	2.016	2.566	0.856
20	*Bacteroidetes* bacterium (HQ821475)	JN936831	98	1.016	2.499	0.868
21	Uncultured bacterium (EU746709)	JN936832	95	0.853	2.426	0.583
22	Uncultured *Crenothrix sp.* (DQ984191)	JN936833	99	2.236	2.527	1.197
23	*Methanotrophic proteobacterium* (EF587743)	JN936834	96	2.412	3.763	2.146
24	Uncultured *beta proteobacterium* (HM854317)	JN936835	98	2.213	3.312	2.361
25	Uncultured *proteobacterium* (GU472649)	JN936836	96	1.152	2.677	1.048
26	*Acidobacteria* bacterium (GU257774)	JN936837	97	2.512	1.011	2.267
27	*Caldilineaceae* bacterium (FM176936)	JN936838	93	2.476	0.874	0.963

Lane A_1: fresh sludge sample of Plant A; Lane B: fresh sludge sample of Plant B; Lane C: stale sludge sample of Plant B. Clone numbers corresponding to the bands marked in Figure 1.

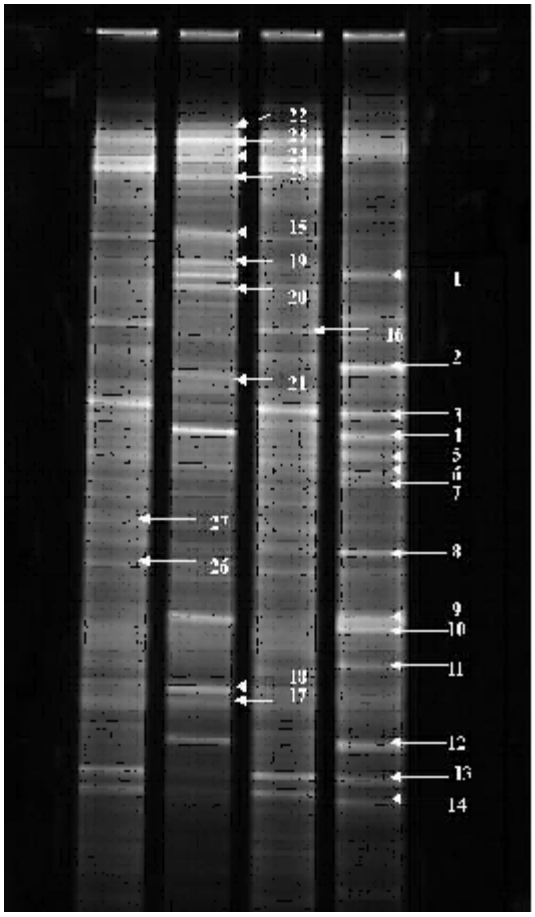

Fig. 1. DGGE band profiles of V3 fragments of 16SrDNA amplified using the total genomic DNA extracted from sludge of drinking water. Lane A_1, A_2: fresh sludge sample of Plant A; Lane B: fresh sludge sample of Plant B; Lane C: stale sludge sample of Plant B

Sequencing of DGGE bands and phylogenetic analysis

A neighbor-joining phylogenetic tree was constructed to visualize the relationships between the sequences. The species of the obtained sequences in DGGE profiles were determined by comparing the closest sequences with those in the National Center for Biotechnology Information (NCBI) database (Liu *et al*., 2011), revealing that the average sequence similarity to the highest matches was above 93% for the selected DGGE bands (Table 2).As a result of phylogenetic analysis (Figure 2 and Table 3), all the sequences could be divided in 9 phyla as follows: '!*Proteobacteria* (eg. bands 5, 6, 9, 16, 23, 24, 25); a! *Acidobacteria* (eg. bands 3, 26); b! *Fibrobacteres* (eg. bands 7, 12); c! *Chloroflexi* (eg. bands 8, 13, 14, 18, 27); d! *Bacteroidetes* (eg. bands 1, 15, 19, 20); e! *Firmicutes* (eg. band 17); f! *Cyanobacteria* (eg. band 11); g! *Sheathe bacteria* (eg. band 22); h! *Verrucomicrobia* (eg. bands 2, 4).

Proteobacteria

DGGE bands 16, 23, 24, 25 had the same nearest phylogenetic neighbor *Proteobacterium* and similarities were 96%, 96%, 98% and 96% respectively, band 9 was more similar to *Escherichia coli* (GU415870, 95%) which was the most common species in the drinking water, and these five bands were distinctly related to species from the genus *Gamma-proteobacteria*, these populations were all gram-negative bacilli and had been considered to be related to phosphate removal (Kavanaugh *et al*., 1994), most of them which enjoyed living in intestinal tract were identified as pathogens. Bands 5 and 6 showed the highest nucleotide similarities to the family of *Rhodocyclaceae* bacterium, which belonged to *Beta-proteobacteria* with the same sequence homologies (97%), Bacteria from the *Beta-proteobacteria* subclass had already been observed by FISH to be dominant in activated sludge communities (Manz *et al*., 1994). Given their numerical dominance, it is likely that representatives of this group of the bacterium played important roles in aspects of sludge such as decomposition of organic materials, removal of nutrients, and formation of floc structure.

Bacteroidetes

Populations related to *Sphingobacteria* represented bands 1, 15 and 20 which showed nucleotide similarities from 96% to 98% respectively, band 1 was more similar to *Bacteroidetes* bacterium (EF636477) which was isolated from the bacterial community of excess activated sludge during heat-treatment (Yan *et al*., 2008), band 15 (similarities with EU283360 which was selected in activated sludge from a consecutively aerated submerged membrane bioreactor treating domestic wastewater) (Du *et al*., 2008) formed a common lineage to the same taxon; band 19 showed 93% (FJ764434, NCBI) similarity to *Flavobacteriaceae* bacteriumÿboth *Sphingobacteria* and *Flavobacterium* were related to *Bacteroidetes* which were always detected in aquatic environment and some of them were

Table 3. Classification of species in drinking water sludge sample

Phylum	Class	Order	Family	Genus	Band No.[a]
Bacteroidetes	*Sphingobacteria*	*Sphingobacteriales*	unclassified	unclassified	1, 15, 20
	Flavobacteria	*Flavobacteria*	*Flavobacteriaceae*	*Flavobacterium*	19
Proteobacteria	*Betaproteobacteria*	*Rhodocyclales*	*Rhodocyclaceae*	*Rhodocyclus*	5, 6,
	Gammaproteobacteria	*Methylococcales*	*Methylococcaceae*	*Methylothermus*	16, 23, 24, 25
	Gammaproteobacteria	Enterobacteriales	*Enterobacteriaceae*	*Escherichia coli*	9
Chloroflexi	*Chloroflexi*	*Chloroflexales*	*Chloroflexaceae*	unclassified	8, 13, 14
	Caldilineae	*Caldilineales*	*Caldilineaceae*	*Caldilinea*	27
	Anaerolineae	*Anaerolineales*	*Anaerolineaceae*	unclassified	18
Acidobacteria	*Acidobacteria*	unclassified	unclassified	unclassified	3, 26
Verrucomicrobia	*Verrucomicrobiae*	*Verrucomicrobiales*	*Verrucomicrobiaceae*	*Verrucomicrobium*	2, 4
Fibrobacteres	*Fibrobacteres*	unclassified	unclassified	unclassified	7, 12
Cyanobacteria	*Cyanobacteria*	*Chroococcales*		*Cyanobacterium*	11
Firmicutes	*Clostridia*	*Clostridiales*	*Eubacteriaceae*	*Eubacterium*	10, 17
Others				*Crenothrix*	22

a: the number was corresponded to the bands marked in Figure 1.

pathogen. The phylum *Bacteroides* is well-known for comprising some of the bacteria present in anaerobic digesters, and its main role in the fermentation system is to break down macromolecules such as cellulose, protein, fiber, starch and chitin (Ponpium *et al*., 2000).

Chloroflexi

The sequences of band 8, 13 and 14 formed a coherent cluster related to the class of *Chloroflexi* with the higher sequence homologies (99%, 100% and 98% respectively), band 18 was more similar to *Anaerolineae* bacterium (EF491509, similarity 94%) and band 27 showed 93% (FM176936) sequence similarity to *Caldilineaceae* bacterium, all of the five bands had closer relationships with the phylum of *Chloroflexi* which was formerly known as green non-sulfur bacteria and has been recognized as a typical bacterial cluster containing a number of diverse environmental clones with only a few cultured representati-ves (Kragelund *et al*., 2007). This group of bacterium was facultative anaerobic and gram-negative bacteria and contained a number of diverse environmental clones retrieved from various wastewater treatment plants. They were predominant bacterium which could obtain ene--rgy for their growth though degrading carbohydrates and cellular materials in activated sludge granules from a high-temperature (55!) up-flow anaerobic sludge blanket (UASB) used to treat high-strength organic wastewater (Yamada *et al*., 2005).

Cyanophyta

The sequence of band 11 was related to *cyanobacterium* (FN860124, similarity 98%) which was widespread in soil and aquatic ecosystems, this group of bacteria, possessed dinitrogen-fixing capabilities and microalgae, was known to influence the development or decline of algal blooms which indicated water quality deterioration and might pose a serious threat to animal and human health as several *cyanobacteria* could produce a variety of very potent toxins (Soares *et al*., 2009), therefore, *cyanobacteria* had attracted much attention because they had been frequently recognized as a problematic constituent of water bloom on the surface of lakes/ponds, which might affect the other living creatures by excreting poisonous metabolites. In addition, previous study indicated that *cyanobacteria* had been considered useful for fixing atmospheric nitrogen into ammonia-containing substances, thereby serving as a source of nitrogen supply for aquatic microorganisms (Hori *et al*., 2002).

Acidobacteria

Species related to *Acidobacteria* bacterium showed band 3 which represented 95% homologies and band 26 showed 97% similarity to GU257774 mentioned from membrane biofilms in a submerged polyviny chloride membrane bioreactor (Xia *et al*., 2010). This group of bacteria which is known as a degrader with high organic materials degradation activity involved in pollutant degradation suggested the importance of such communities for drinkingwater treatment.

Other Populations

Bands 7 and 12, with the same sequence homologies (similarity 99%), were related to *Fibrobacteres* bacterium which were gram-

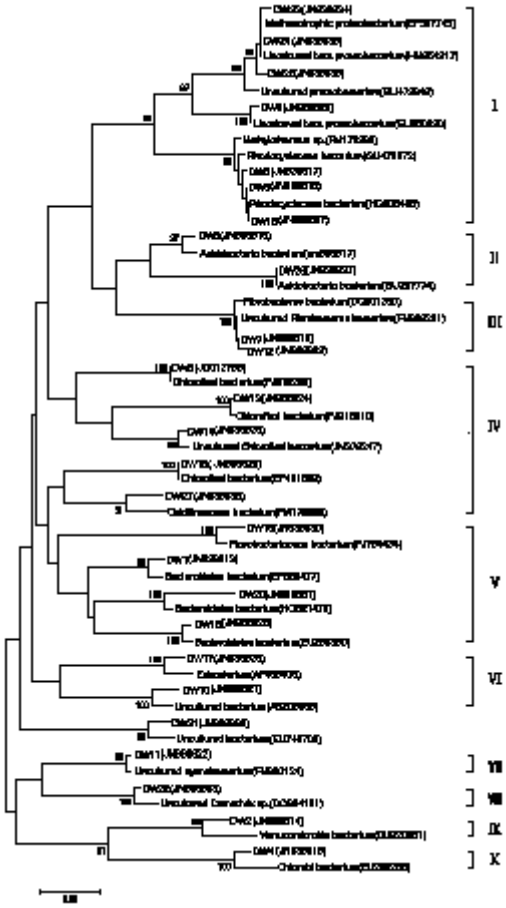

Fig. 2. Phylogenetic positions of the V3-16SrDNA region sequences. Clones corresponding to the bands marked in Figure 1

negative bacterium and could resolve cellulose. Band 2 (CU920931, similarity 95%) and band 4 (EU299285, similarity 93%) (Jangid *et al*., 2010) formed a lineage with *Verrucomicrobia* bacterium which mainly lived in soil, water and human excreta, therefore, they probably belonged to one species. Band 22 and GU257774 (similarity 97%) formed a common lineage to the same genus of *Crenothrix* which was belonged to *Sheathe* bacteria and likely to lived in flow of fresh water which contained rich organic. In addition, both bands 10 and 21 had high similarities (98% and 95%) to reference strains found in the NCBI database but were uncultured, band 10 was more to band 17 which was belonged to *Eubacterium* (similarity 98%) according to the phylogenetic tree, so they may formed a coherent cluster to *Firmicutes* which were weak and could not resist strong shear imposed on them, unlike *Beta-proteobacteria* or *Gamma-proteobacteria* (Larsen *et al*., 2008), but band 21 were difficult to be classified (Figure 2). All the clones clustered with various sequences in the National Center for Biotechnology Information (NCBI) database retrieved from activated sludge, lake, water, soil, contaminated environments, and so on.

The differences of microbial community structure in different drinking water sludge

According to Table 4, the common predominating phyla in different drinking water sludge included *Proteobacteria*, *Bacteroidete*s and *Chloroflexi*, which were also common species in wastewater treatment systems, but certain populations were specific, such as *Acidobacteria* in Lane A_1 and *Verrucomicrobia* in Lane B. As shown in Figure 1 and Table 3, the bacterial community structures of fresh sludge samples in different plants (between Lane A_1 and Lane B) were similar and a number of common species such as *Sphingobacteria* (band 15), *Eubacterium* (band 17), *Anaerolineae* (band 18), *Methylococcaceae* (bands 23, 24 and 25) were lived in, but the quantity of them had more or less different. For example, the quantity of band 15 in Lane A_1 and Lane B was nearly the same with the OD value 2.128, 2.513 respectively, the OD value of band 18 in Lane A_1 was 1.121 but it was much higher in Lane B (2.911). A few species were also particular for one of them due to the standing time and the water quality. For Lane A_1, *Chloroflexaceae* (bands 13 and 14) and *Acidobacteria* (band 3) were the prominent species, but more numbers of *Sphingobacteria* (band 1) *Verrucomicrobiaceae* (band 4) and *Firmicutes* (band 12) were existed in Lane B. Differences in raw water quality are likely to be the reason for the differences in microbial community structure of different plant sludge observed in the present study.

Compared to Lane B, Lane C also had the same species (eg. *Bacteroidetes*, *Proteobacteria* and so on), but *Chloroflexaceae* (bands 8, 13, 14) and *Verrucomicrobia* (band 2) were also abundant populations attributed to the different place times in the same plant. This effect on bacterial community structure can be explained by the dissolved oxygen and the organic materials of the sludge gradually reduced as the residence time increased.

The comparison of microbial community structure between drinking water sludge and wastewater sludge

Compared to the microorganism composing of activated sludge in waste water treatment plants, *Proteobacteria*, *Bacteroidete*s and *Chloroflexi* were the most common abundant populations on phylum level and other species such as *Verrucomicrobia, Firmicutes* and *Acidobacteria* might become dominating ones in certain special environments (Hu *et al*., 2012). Furthermore, as shown in Table 5, many differences were existed between drinking water sludge and wastewater sludge, for example, the number of *Bacteroides*, *Rhodocyclus*, *Cyanobacterium* and *Chloroflexus* were seen as the abundant species in drinking water sludge, but in activated sludge of waste water, they were less in certain special wastewater systems and *Zoogloea*, *Comamonas*, *Alcaligenes* and *Micrococcus* were counted as the prominent species in it according to the large number of their individuals. In addition, there were also some common species such as *Flavobacterium*, *Proteobacterium, Escherichia coli* and so on between drinking water sludge and wastewater sludge (Moura *et al*., 2009). However, the bacterial community structure of the sewage sludge treatments differed from that of the drinking water treatments. This effect on bacterial community structure can be explained by the different treatment process and the inflow water quality such as the concentrations and types of DOC and heavy metals, the concentrations of the nitrogen or

Table 4. The differences of microbial community structures between Plant A and B

	Lane A_1	Lane B
Special abundant bands	*Acidobacteria*	*Verrucomicrobi, Fibrobactere, Firmicutes*
Common abundant bands	*Bacteroidetes* , *Proteobacteria, Chloroflexi*	

Lane A_1: fresh sludge sample of Plant A; Lane B: fresh sludge sample of Plant B

Table 5. Comparison of sludge composition between drinking water treatment plants and wastewater treatment plants

Types of sludge	Special abundant species	Common abundant species
Drinking water sludge	*Bacteroides*, *Rhodocyclus*, *Cyanobacterium, Chloroflexus*	*Flavobacterium*, *Proteobacterium, Escherichia coli* and so on.
Wastewater sludge	*Zoogloea*, *Comamonas*, *Alcaligenes, Micrococcus*	

phosphorus compounds and the DO concentrations in the water.

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