Letter to the Editor

Bacterial Communities Associated with An Occurrence of Colored Water in An Urban Drinking Water Distribution System*

WU Hui Ting[1,2,△], MI Zi Long[1,2,△], ZHANG Jing Xu[3], CHEN Chao[1,2], and XIE Shu Guang[3,#]

This study aimed to investigate bacterial community in an urban drinking water distribution system (DWDS) during an occurrence of colored water. Variation in the bacterial community diversity and structure was observed among the different waters, with the predominance of *Proteobacteria*. While *Verrucomicrobia* was also a major phylum group in colored water. *Limnobacter* was the major genus group in colored water, but *Undibacterium* predominated in normal tap water. The coexistence of *Limnobacter* as well as *Sediminibacterium* and *Aquabacterium* might contribute to the formation of colored water.

The drinking water distribution system (DWDS) is mainly composed of iron and steel pipes in many countries that are subject to corrosion during long-term usage. Corrosion scales can be severe in the old iron pipes in this system, which can adversely affect drinking water quality and entail some adverse consequences[1-2]. It has been recognized that iron release can be influenced by a variety of water quality parameters[1,3-4]. In addition, microorganisms might also play an important role in colored water events as they can accelerate the process of iron corrosion[2-3]. However, information on microbial communities in the DWDS associated with the occurrence of colored water is still scant. Therefore, the main objective of the present study was to investigate bacterial communities in an urban DWDS during an occurrence of colored water.

Tap water samples were collected from the DWDS in a city of Southeast China. The DWDS was fed with the treated surface water (with treatments of rapid mixing, flocculation, sedimentation, sand filtration and chlorination). It was mainly composed of cast iron pipes. At time of sample collection, the majority of urban areas reported the occurrence of colored water, but the water quality in some areas was still normal, without perceptible color. Water samples were collected from three colored water points A, B and C, and a normal point D (as a reference point). All water samples were immediately processed after collection. The physicochemical features of the four water samples are shown in Table S1.

For analysis of aquatic bacterial community, water samples (5 L) were filtered through 0.22-μm pore-size membranes (diameter 50 mm; Millipore, USA). The membrane filters stored at -20 °C for further molecular analysis. DNA samples were extracted using the E.Z.N.A.® Water DNA kit (Omega, USA) according to the manufacturer's protocol. Bacterial 16S rRNA genes were amplified using primers 27F (5'-GAGTTTGATCMTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). Chimera-free sequences with similarity of more than 98% were assigned as operational taxonomic units (OTUs). OTUs, rarefaction curves, and Shannon diversity index were obtained using the DOTUR program[5]. The Ribosomal Database Project analysis tool 'classifier' was used to assign the taxonomic identities of the obtained bacterial sequences[6]. Phylogenetic tree of the selected sequences was constructed using the MEGA software version 4.0 with a neighbor-joining algorithm by performing a boostrap analysis with 1 000 replicates[7]. In addition, Pearson's correlation analysis of the bacterial community structure with the determined physicochemical parameters was conducted using the SPSS 20.0 software. The 16S rRNA sequences obtained in this study were submitted to GenBank under accession numbers KF514896-KF514971

doi: 10.3967/bes2014.099

*This work was financially supported by State Environmental Protection Key Laboratory of Microorganism Application and Risk Control (No. MARC2012D010), National Water Special Program (No. 2012ZX07404-002), and International Science & Technology Cooperation Program of China (No. 2010DFA91830).

1. State Environmental Protection Key Laboratory of Microorganism Application and Risk Control (MARC), Tsinghua University, 100084 Beijing, China; 2. School of Environment, Tsinghua University, Beijing 100084, China; 3. State Key Joint Laboratory of Environmental Simulation and Pollution Control, College of Environmental Sciences and Engineering, Peking University, Beijing 100871, China

(library with Sample A), KF514972-KF515038 (library with Sample B), KF515039-KF515114 (library with Sample C), and KF515115-KF515197 (library with Sample D).

As shown in Table S2, 7, 27, 9, and 13 OTUs were obtained in the four bacterial clone libraries constructed with Sample A, Sample B, Sample C, and Sample D, respectively. The OTU-based Shannon diversity index in Sample B was much higher (2.95) than that in the other three tap water samples (0.55-1.35). The rarefaction curves for Samples A, C, and D leveled off completely, indicating that the bacterial communities in these three water samples were well sampled, with a low bacterial community diversity (Figure S1). However, the rarefaction curve for Sample C did not approach a plateau, suggesting that further sequencing would have resulted in more OTUs. These results also illustrated the differences in community diversity among the four water samples. The bacterial phylum compositions of the four water samples are shown in Figure 1. In this study, five phyla were identified in these samples, including that *Proteobacteria*, *Bacteroidetes*, *Verrucomicrobia*, *Planctomycetes,* and *Spirochaetes*. *Proteobacteria* was the only phylum detected in Samples A and D, and it also predominated in Samples B and C. The

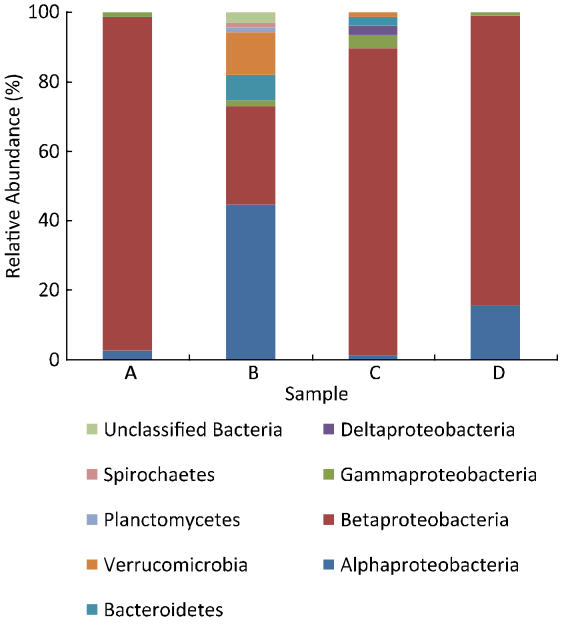

Figure 1. Comparison of the quantitative contribution of the sequences affiliated with different phyla and subphyla to the total number of sequences in Samples A-D. Sequences not classified to any known phylum are included as unclassified bacteria.

major bacterial group (with relative abundance no less than 10%) in both Sample A and Sample C was *Betaproteobacteria* (96.1% or 88.2%). Sample D was mainly represented by *Betaproteobacteria* (83.1%) and *Alphaproteobacteria* (15.7%). Sample B was mainly composed of *Alphaproteobacteria* (44.8%), *Betaproteobacteria* (28.4%), and *Verrucomicrobia* (11.9%). Therefore, a shift in the composition of major bacterial groups occurred in the different tap waters. These results also confirmed the bacterial growth in the DWDS.

Table 1 shows the distribution of the 22 known bacterial genera detected in the four tap water samples. At the genus level of taxonomic classifications, differences of bacterial community structure among the four samples were more evident. For Samples A, C, and D, most of sequences

Table 1. Distribution of the Sequences Affiliated with the Identified Genera in Samples A-D

Phylogenetic Affiliation	Sample A	Sample B	Sample C	Sample D
Alphaproteobacteria				
Sphingobium	-	-	-	5
Novosphingobium	-	8	-	2
Caulobacter	-	1	-	-
Hyphomicrobium	-	1	-	-
Methylobacterium	-	1	-	-
Afipia	-	-	-	2
Bosea	-	-	-	1
Rhodocista	-	-	-	2
Betaproteobacteria				
Limnobacter	67	3	66	-
Methyloversatilis	1	1	-	1
Aquabacterium	-	3	-	7
Acidovorax	-	-	-	3
Curvibacter	-	6	-	1
Undibacterium	-	-	-	56
Gammaproteobacteria				
Pseudomonas	1	-	-	-
Caedibacter	-	2	-	-
Legionella	-	-	-	1
Bacteroidetes				
Sediminibacterium	-	2	1	-
Hydrotalea	-	1	-	-
Pedobacter	-	1	-	-
Planctomycetes				
Planctomyces	-	1	-	-
Spirochaetes				
Leptonema	-	1	-	-
Total	69	32	67	81

Note. -, not detected.

could be related to known bacterial genera. In contrast, for Samples B, less than half of the total sequences (32/67) could be classified at genus level. There was no genus shared among all the samples. Moreover, *Limnobacter* predominated in Samples A and C, but were not detected or detected with quite a low abundance in the other two samples. *Undibacterium* was the predominant component in Sample D, but was not found in the other three samples.

Li and his colleagues investigated the bacterial communities in three colored waters and normal water from a real DWDS in Beijing, China. They indicated that the Shannon diversity indices for all the tap water samples ranged from 3.15 to 4.02[3]. In this study, the water with the highest ferrous level and turbidity (Sample A) showed the lowest bacterial diversity (Shannon index=0.55), while the water with the second highest ferrous level and turbidity (Sample B) showed a largest bacterial diversity (Shannon index=2.95). In addition, Pearson's correlation analysis indicated that Shannon diversity index did not show any significant correlation with each of the measured physicochemical properties (P>0.05) (data not shown). However, in this study, only four water samples were detected, which might frustrate the efforts to find the links between water physicochemical properties and bacterial diversity. Moreover, the bacterial diversity might be regulated by water age and other factors.

To date, microbial communities in the DWDS associated with the occurrence of colored water remains largely unknown. Only Li and his colleagues investigated the bacterial communities in colored waters of a real DWDS. They found that *Proteobacteria* predominated in three red water samples, with other minor bacterial groups including *Bacteroidetes*, *Actinobacteria* and *Planctomycetes*, while both *Proteobacteria* and *Bacteroidetes* were the major bacterial groups in a normal water (without perceptible color and turbidity). Their studies also indicated that *Betaproteobacteria* and *Alphaproteobacteria* were the dominant bacterial group in two red waters, but *Gammaproteobacteria* and *Betaproteobacteria* were the major components in the other one red water[3]. In this study, the proportions of *Alpha-*, *Beta-*, and *Gammaproteobacteria* varied among the three colored waters. The composition of major proteobacterial classes and their relative abundance in colored waters were not in agreement with the results from others' previous study[3]. Therefore, the bacterial community structure in colored water might be dependent on both location of DWDS and sampling site. However, Pearson's correlation analysis indicated that the relative abundance of major bacterial groups was not significantly correlated with the determined physicochemical properties (P>0.05) (Table S3). More intensive sampling activity will be necessary in order to elucidate the heterogeneity of bacterial community structure in colored waters. Moreover, bacterial community structure might be regulated by water age and other factors. *Verrucomicrobia* is widely present in terrestrial and aquatic habitats, although the role of verrucomicrobial species in the environment remains poorly understood. In this study, the abundance of *Verrucomicrobia* was found in the tap water.

Iron bacteria are capable of efficiently oxidizing and depositing ferrous ions in water, and they are ubiquitous in various types of natural waters. To date, a variety of iron-oxidizing bacteria from diverse genera have been isolated, and among them members of genera *Gallionella*, *Sphaerotilus*, *Leptothrix*, *Crenothrix,* and *Siderocapsa* have been well studied[3-4]. Iron-oxidizing bacteria could be an important contributor to corrosion of the water distribution system[3-4]. Abundance of ferrous iron in colored water can create favorable conditions for the growth of iron-oxidizing bacteria in DWDS[3]. In events of colored water, some neutrophilic iron oxidizers, such as *Gallionella* spp. and *Leptothrix ochracea*, were observed[8]. Based on clone library analysis, Li and his colleagues found the high abundance of iron-oxidizing bacteria in three colored water samples, including neutrophilic microaerobic *Gallionella* and *Sideroxydans*, acidophilic *Acidothiobacillus*, and anaerobic denitrifying *Thermomonas*, while *Gallionella* comprised 18.7% to 28.6% of the total of their obtained clones. Their quantitative PCR analysis further confirmed the high abundance of *Gallionella* in the colored water samples, and hence suggested an important role played by iron-oxidizing bacteria *Gallionella* in the formation of colored water[3]. However, interestingly, all of the detected sequences in this study were not related to the previously reported iron-oxidizing bacteria in colored water.

Sediminibacterium sp. has been linked to iron oxidization, and its abundance has been detected in the corrosion scales of cast iron pipes in a reclaimed water distribution system[2]. Unfortunately, the parameter affecting the abundance of *Sediminibacterium* sp. in the environment remains in

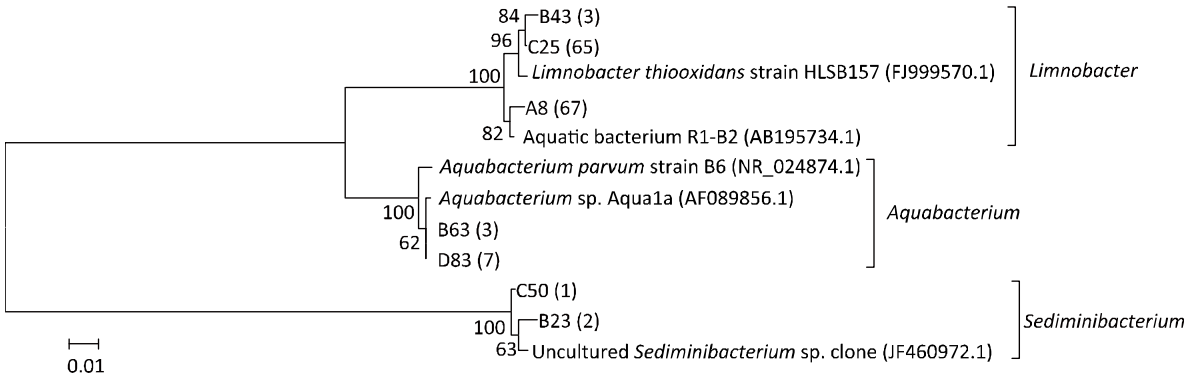

Figure 2. Phylogenetic tree of representative 16S rRNA gene sequences assigned with putative genera *Limnobacter*, *Aquabacterium,* and *Sediminibacterium* and reference sequences from GenBank. The obtained sequences beginning with 'A', 'B', 'C', and 'D' were referred to sequences recovered from Sample A-D, respectively. The bold number in parentheses represents the numbers of the sequences in the same genus. Numbers at the nodes indicate the levels of bootstrap support based on neighbor-joining analysis of 1 000 resampled datasets. Bootstrap values <50 are not shown. The bar represents 1% sequence divergence.

this study, three *Sediminibacterium*- related sequences were detected in colored waters. They were closely related (with 99% or 100% similarity) to an uncultured *Sediminibacterium* species (JF460972.1) retrieved from a DWDS (Figure 2). In addition, Pearson's correlation analysis indicated that the relative abundance of *Sediminibacterium* had a negative significant correlation with DO (P<0.05) (Table S4).

Aquabacterium species are also known as iron-oxidizing bacteria[9] and have been isolated from the drinking water system[10]. In this study, three *Aquabacterium*-like sequences were detected in two colored waters and seven in normal water. These sequences were closely related (with 99% similarity) to an *Aquabacterium* strain isolated from the drinking water biofilm (AF089856.1), or another one from the Berlin drinking water system[10] (Figure 2). This was the first report on the presence of *Aquabacterium* in colored waters. However, the relative abundance of *Aquabacterium* did not show any significant correlation with each of the measured physicochemical properties (P>0.05) (Table S4).

Microorganisms from genus *Limnobacter* are known as sulfur-oxidizing bacteria[2]. Sulfur-oxidizing bacteria could convert ferrous sulfide to sulfuric acid, releasing ferrous iron in waters, which in turn was utilized by iron-oxidizing bacteria[3]. In this study, *Limnobacter*-related sequences were detected with a high abundance in colored waters. To the authors' knowledge, this was the first report on the dominance of *Limnobacter* in drinking water. They had 98% or 99% similarities to a *Limnobacter thiooxidans* strain (FJ999570.1) isolated from South China Sea (Figure 2). Therefore, the dominance of *Limnobacter* might facilitate the iron release from pipe materials into waters. The presence of *Aquabacterium* as well as *Sediminibacterium* might accelerate the precipitation of iron oxides by converting ferrous iron to ferric one. The coexistence of sulfur-oxidizing bacteria and iron-oxidizing bacteria might contribute to the formation of colored water.

In conclusion, difference in the diversity and structure of bacterial communities occurred in colored and normal tap waters. *Proteobacteria* was the predominant component in the DWDS during the occurrence of colored water. The coexistence of *Limnobacter* as well as *Sediminibacterium* and *Aquabacterium* might promote the formation of colored water.

All the supplementary tables and figure can be found in the website of www.besjournal.com

△The first two authors contribute equally to this paper.

#Correspondence should be addressed to XIE Shu Guang, Tel: 86-10-62751923, E-mail: xiesg@pku.edu.cn

Biographical notes of the first authors: WU Hui Ting, female, born in 1990, master student, majoring in drinking water treatment. MI Zi Long, male, born in 1988, PhD candidate, majoring in drinking water treatment.

Received: April 15, 2014;

Accepted: June 18, 2014

REFERENCES

1. Sarin P, Snoeyink VL, Bebee J, et al. Iron release from corroded iron pipes in drinking water distribution systems: effect of dissolved oxygen. Water Res, 2004; 38, 1259-69.
2. Wang HB, Hu C, Hu XX, et al. Effects of disinfectant and biofilm on the corrosion of cast iron pipes in a reclaimed water distribution system. Water Res, 2012; 46, 1070-8.
3. Li D, Li Z, Yu JW, et al. Characterization of bacterial community structure in a drinking water distribution system during an occurrence of red water. Appl Environ Microbiol, 2010; 76, 7171-80.
4. Wang Y. Characteristics of iron stability and red water control in drinking water distribution systems. Ph. D Dissertation, Tsinghua University, 2009. (In Chinese)
5. Schloss PD, Handelsman J. Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. Appl Environ Microbiol, 2005; 71, 1501-6.
6. Wang Q, Garrity GM, Tiedje JM, et al. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol, 2007; 73, 5261-7.
7. Tamura K, Dudley J, Nei M, et al. MEGA4, molecular evolutionary genetics analysis, MEGA software version 4.0. Mol Biol Evol, 2007; 24, 1596-9.
8. Volk C, Dundore E, Schiermann J, et al. Practical evaluation of iron corrosion control in a drinking water distribution system. Water Res, 2000; 34, 1967-74.
9. Straub KL, Schonhuber WA, Buchholz-Cleven BEE, et al. Diversity of ferrous iron-oxidizing, nitrate-reducing bacteria and their involvement in oxygen-independent iron cycling. Geomicrobiol J, 2004; 21, 371-8.
10. Kalmbach S, Manz W, Wecke J, et al. *Aquabacterium* gen. nov., with description of *Aquabacterium citratiphilum* sp. nov., *Aquabacterium parvum* sp. nov. and *Aquabacterium commune* sp. nov., three in situ dominant bacterial species from the Berlin drinking water system. Int J Syst Bacteriol, 1999; 49, 769-77.

Supplementary Information

Table S1. Physicochemical Features of The Four Tap Water Samples

Sample	Total Ferrous (mg/L)	Turbidity (NTU)	Residual Chlorine (mg/L)	pH	DO (mg/L)	UV_{254}	DOC (mg/L)	NH_4^+-N (mg/L)	NO_2^--N (mg/L)	NO_3^--N (mg/L)
A	138.2	338	0.43	7.10	7.91	0.034	0.72	0.02	0.117	2.962
B	23.2	47.8	0.03	7.10	4.56	0.029	0.96	0.027	0.007	2.902
C	9.4	16.5	0.03	7.10	6.32	0.030	1.44	0.026	0.008	2.984
D	0.1	0.4	0.05	7.10	6.90	0.032	1.28	0.03	0.041	3.061

Table S2. OTU-based Community Richness and Diversity Indices for Samples A-D.

Sample	No. of Sequences	OTUs	Shannon Index
A	76	7	0.55
B	67	27	2.95
C	76	9	0.7
D	83	13	1.35

Table S3. Pearson's Correlation Coefficients Describing the Relationship Between Water Characteristics and the Relative Abundance of Phylum Groups. Pearson's Correlation Analysis was Performed using SPSS 20.0 Software

Bacterial Groups	Total Ferrous	Turbidity	Residual Chlorine	DO	UV_{254}	DOC	NH_4^+-N	NO_2^--N	NO_3^--N
Alphaproteobacteria	-0.38	-0.39	-0.47	-0.82	-0.60	-0.17	0.51	-0.49	-0.45
Betaproteobacteria	0.29	0.31	0.44	0.93	0.74	0.19	-0.31	0.56	0.74
Gammaproteobacteria	-0.34	-0.35	-0.36	-0.05	-0.38	0.70	0.05	-0.45	0.07
Deltaproteobacteria	-0.34	-0.35	-0.36	-0.05	-0.38	0.70	0.05	-0.45	0.07
Bacteroidetes	-0.36	-0.38	-0.53	-0.96*	-0.88	-0.01	0.28	-0.69	-0.78
Verrucomicrobia	-0.26	-0.28	-0.41	-0.92	-0.75	-0.21	0.26	-0.55	-0.78
Planctomycetes	-0.20	-0.22	-0.36	-0.88	-0.68	-0.29	0.25	-0.47	-0.76
Spirochaetes	-0.20	-0.22	-0.36	-0.88	-0.68	-0.29	0.25	-0.47	-0.76

Note. *Correlation is significant at the 0.05 level.

Table S4. Pearson's Correlation Coefficients Describing the Relationship Between Water Characteristics and the Relative Abundance of Genus Groups. Pearson's Correlation Analysis was Performed using SPSS 20.0 Software

Bacterial Groups	Total Ferrous	Turbidity	Residual Chlorine	DO	UV_{254}	DOC	NH_4^+-N	NO_2^--N	NO_3^--N
Sphingobium	-0.44	-0.42	-0.29	0.23	0.23	0.37	0.64	-0.03	0.85
Novosphingobium	-0.31	-0.32	-0.43	-0.88	-0.66	-0.22	0.39	-0.50	-0.62
Caulobacter	-0.20	-0.22	-0.36	-0.88	-0.68	-0.29	0.24	-0.47	-0.76
Hyphomicrobium	-0.20	-0.22	-0.36	-0.88	-0.68	-0.29	0.24	-0.47	-0.76
Methylobacterium	-0.20	-0.22	-0.36	-0.88	-0.68	-0.29	0.24	-0.47	-0.76
Afipia	-0.44	-0.42	-0.29	0.23	0.23	0.37	0.64	-0.03	0.85
Bosea	-0.44	-0.42	-0.29	0.23	0.23	0.37	0.64	-0.03	0.85
Rhodocista	-0.44	-0.42	-0.29	0.23	0.23	0.37	0.64	-0.03	0.85
Limnobacter	0.57	0.57	0.56	0.55	0.38	-0.10	-0.78	0.43	-0.11
Methyloversatilis	0.34	0.35	0.32	-0.09	0.26	-0.75	-0.07	0.38	-0.23
Aquabacterium	-0.68	-0.66	-0.60	-0.28	-0.22	0.37	0.87	-0.39	0.51
Acidovorax	-0.44	-0.42	-0.29	0.23	0.23	0.37	0.64	-0.03	0.85
Curvibacter	-0.27	-0.29	-0.41	-0.89	-0.67	-0.25	0.34	-0.49	-0.67
Undibacterium	-0.44	-0.42	-0.29	0.23	0.23	0.37	0.64	-0.03	0.85
Pseudomonas	**0.99***	**0.99***	**0.99***	0.71	0.83	-0.79	-0.93	**0.95***	-0.15
Caedibacter	-0.20	-0.22	-0.36	-0.88	-0.68	-0.29	0.24	-0.47	-0.76
Legionella	-0.44	-0.42	-0.29	0.23	0.23	0.37	0.64	-0.03	0.85
Sediminibacterium	-0.37	-0.40	-0.54	**-0.95***	-0.89	0.02	0.28	-0.71	-0.77
Hydrotalea	-0.20	-0.22	-0.36	-0.88	-0.68	-0.29	0.24	-0.47	-0.76
Pedobacter	-0.20	-0.22	-0.36	-0.88	-0.68	-0.29	0.24	-0.47	-0.76
Planctomyces	-0.20	-0.22	-0.36	-0.88	-0.68	-0.29	0.24	-0.47	-0.76
Leptonema	-0.20	-0.22	-0.36	-0.88	-0.68	-0.29	0.24	-0.47	-0.76

Note. *Correlation is significant at the 0.05 level.

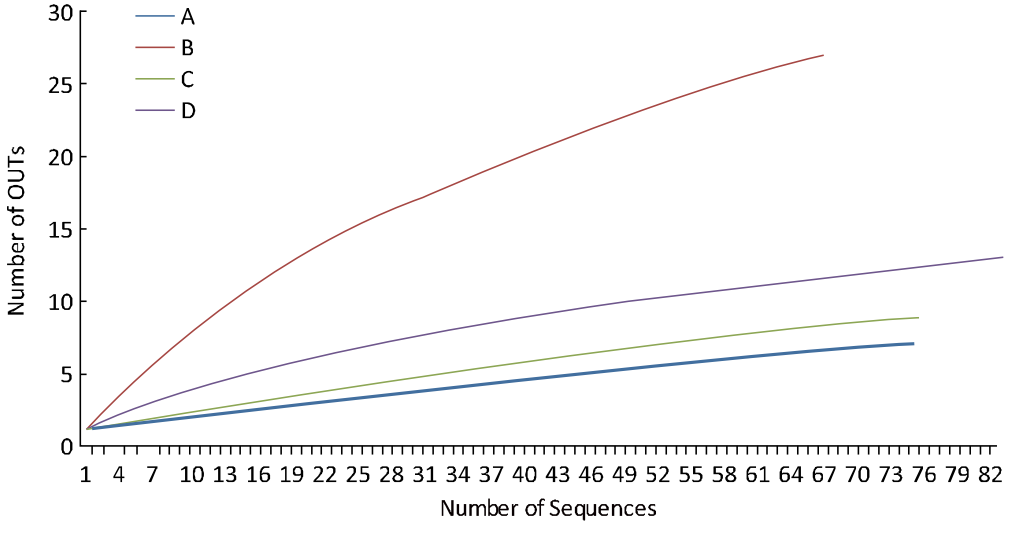

Figure S1. Rarefaction curves of OTUs in Samples A-D evaluated by 2% sequence variation.