보건학석사 학위 논문

Characterization of changes in cultured house dust-borne bacterial communities and diversities by biocides application

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Abstract

Characterization of changes in cultured house dust-borne bacterial communities and diversities by biocides application

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Bacteria are ubiquitous in indoor environments, and some species may cause clinical symptoms in human. To control indoor bacteria, biocides are often used. However, there are concerns about biocide utilization such as direct toxicity to human, emergence of drug-resistant bacteria, and changes in indoor bacterial communities and diversities. This study aimed to examine changes in cultured house dust-borne bacterial communities and diversities by application of three types of biocides, including triclosan, copper (II) sulfate ($CuSO_4$), and benzalkonium chloride (BAC). Eighteen house dust samples, collected from one student dormitory were used. This study quantified changes of bacterial counts by a conventional growth-based method and analyzed bacterial communities and diversities, utilizing operational taxonomic units (OTUs) data obtained from the next-generation sequencing (NGS) based on 16S rDNA.

The results showed that the biocide concentrations recommended by European Commission and U.S. FDA as the maximum safe and effective concentrations in consumer products could effectively reduce the culturable bacteria with no colony detected on the biocide-containing nutrient plates. The 1/10000 diluent biocides could also reduce the culturable dust-borne bacteria by 37% for

BAC, 50% for triclosan, and 63% for $CuSO_4$. In addition, the bactericidal efficacies appeared to be taxon-dependent. BAC was more effective against *Proteobacteria* with the average reduction ratio of 96% (1/10000 diluent), whereas triclosan removed 100% of *Actinobacteria* (1/100 diluent). $CuSO_4$ had similar reduction ratios to the three main detected phyla of *Proteobacteria* (100%), *Actinobacteria* (97%), and *Firmicutes* (100%) (1/100 diluent). To study the bacterial communities' composition and diversities, a total of 8011 operational OTUs based on 97% sequence similarity were studied. The observed OTUs of house dust-borne bacteria were reduced by cultivation with an average reduction ratio of 52%. Compared with the blank (cultured samples without biocides), the observed OTUs in the cultured samples with $CuSO_4$ and triclosan were reduced by 24% and 26%, respectively, indicating reduction in the bacterial species richness in response to exposures to these biocides. The observed OTUs were increased by 1% in the cultured samples with BAC. The bacterial community structures of house dust samples cultured with and without biocides were statistically different ($p < 0.001$; parsimony method) for three biocides. Overall, the findings of this study indicated that changes in house dust-borne bacterial counts, communities, and diversities were dependent on types and concentrations of biocides, providing important insights into how biocides could be used to effectively sterilize bacteria indoors. The bacterial diversities were largely decreased after the biocide exposures, raising a concern about potential human health impacts associated with reductions in indoor bacterial diversities such as allergic diseases and childhood asthma.

Key words: house dust, bacteria, biocide, community, diversity, composition

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1. Introduction

1.1. Health impacts of indoor bacteria

Bacteria are ubiquitous in indoor and outdoor environments (SchultzeLam et. al. , 1996). Some bacteria are essential for human health (Ohland and Macnaughton, 2010) and ecosystems (Naeem and Li, 1997), but certain species are pathogenic, e.g., *Staphylococcus aureus* (Kallen et. al. , 2010), *Streptococcus* (Johansson et. al. , 2010), and *Bacillus anthracis* (Spencer, 2003). *S. aureus* is an opportunistic pathogen, causing skin infections as well as life threatening diseases (Kluytmans et. al. , 1997, Cole et. al. , 2001, Kallen et. al., 2010). *Streptococcus* spp. cause invasive infections with high morbidity and mortality (Johansson et. al., 2010), whereas *B. anthracis* is causative of fatal anthrax diseases (Spencer, 2003).

Owing to the fact that people spend most of their time indoors (Yang et. al. , 2011), indoor bacteria are important from the aspect of human health (Rintala et. al. , 2008, Taubel et. al. , 2009, Dannemiller et. al. , 2015, von Mutius, 2014). For example, bacterial endotoxins, integral components of the outer membrane of Gram-negative bacteria, have a positive association with asthma, allergy and wheezing (Liebers et. al. , 2008, Silverman M H, 1998, Williams et. al. , 2005, Liu, 2002). Additionally, bacteria are thought to be associated with building-related illnesses (Sahlberg et. al. , 2013, Teeuw et. al. , 1994, Godish, 2010). Thus, accurate characterization of indoor bacterial communities and concentrations is essential from the aspect of human health. To assess health risks associated with indoor bacterial communities and concentrations, bacteria in house dusts are occasionally analyzed as a proxy measure for indoor bacterial exposures (Wouters et. al. , 2000, Douwes et. al. , 2006, Frankel et. al. , 2012, Wu et. al. , 2012). House dusts contain large quantities of bacteria with an estimate of 7.2×10^5 cell/mg as described by Karkkainen and colleagues (Karkkainen et. al. , 2010), and pathogenic species are also occasionally found (Baumgardner, 2012, Tejpratap SP, 2011, Kruger et. al. , 2012). Thus, monitoring of bacteria in house dust is important to assess health risks associated with indoor bacterial exposures.

1.2. Bacterial viability and culturability

Traditionally, growth-based methods have been used to quantify culturable house dust-borne bacterial counts for assessment of indoor bacterial exposures (Korthals et. al. , 2008, Torvinen et. al. , 2010). Growth-based methods reply on microbial viability in order to quantify the number of grown microbial colony forming units (CFU). Microbial viability is defined as the ability of cells to form colonies on solid agar plates under suitable growth conditions and/or to proliferate in solutions with sufficient nutrients (Lehtinen, 2007). However, this definition results in ambiguity between viability and culturability (Kell and Young, 2000) since culturability is similarly defined as the ability of a single cell to produce a distinct population (Bogosian and Bourneuf, 2001). Later, the concept of viable but non-culturable (VBNC) bacteria was introduced. VBNC bacteria are in a state of very low metabolic activity but are alive and have the ability to become culturable (Xu et. al. , 1982). Bacteria can be in the VBNC state under environmental stress, such as adverse nutrient, temperature, osmotic, oxygen, and light conditions (Oliver, 2005). Additionally, types of nutrient media utilized can also significantly influence the culturability test results (Davis et. al. , 2005, Chikere C B, 2014). Based on this classification, all culturable cells are considered to be viable, whereas viable cells are not necessarily culturable. Dormancy is defined as a reversible state of metabolic shutdown, which reflects an absence of biological activities (Bar et. al. , 2002). Dormant cells may stop their growth due to injury, or they may be in the VBNC state. In contrast, dead cells are in an irreversible state and lost their ability to regrow (Lehtinen, 2007).

1.3. Biocides for indoor bacteria

Biocides are used to suppress and sterilize microbial agents (SCENIHR, 2009). Examples of biocides used in the indoor environments include triclosan (Schweizer, 2001), benzalkonium chloride (BAC) (Mangalappalli-Illathu and Korber, 2006), and copper (II) sulfate ($CuSO_4$) (Borkow and Gabbay, 2005). Triclosan is contained in consumer products such as soaps, shower gels, and toothpastes (Jones et. al. , 2000, Schweizer, 2001), whereas BAC is included in products such as nasal and cough drops, cleaning agents, and mouthwashes (Graf, 2001, Moran et. al. , 2000). $CuSO_4$ is also used in consumer products such as hair dyes and coloring glass (Matsubara et. al. , 2013).

Bactericidal efficacies and modes of actions are dependent on bacterial species and vary by types of biocides (Russell and McDonnell, 2000, Maillard, 2005). For instance, triclosan interferes with bacterial outer membranes, resulting in cells death due to release of cellular components (Suller and Russell, 2000, SCCP, 2009), whereas $CuSO_4$ binds to microbial proteins, resulting in disruption of protein structures and enzymatic activities (McDonnell and Russell, 1999). BAC has a cationic amphiphilic property that can destabilize bacterial cell membranes. BAC is effective against Gram-positive bacteria, but not effective against most Gram-negative bacteria owing to their layers of inner and outer membranes (Coughlin et. al. , 1983, McDonnell and Russell, 1999, Fazlara and Ekhtelat, 2012). The variability in bactericidal efficacies may result in changes in indoor bacterial communities and diversities when they are used in the indoor environment. Since microbial communities and diversities in house dust are thought to be associated with childhood asthma development (Maier et. al. , 2010, Ege et. al. , 2011, Dannemiller et. al. , 2014, Konya et. al. , 2014), research is needed to characterize how indoor bacterial communities and diversities in response to indoor biocide applications from the aspect of human health.

1.4. Risks of use of biocides indoors

A concern of using biocides in indoor environments lies in their potential human health impacts (Dettenkofer et. al. , 2004). In our daily life, biocides are widely used to ensure personal hygiene, disinfection, and food preservation. Exposures to biocides can occur through ingestion, inhalation, and dermal absorption (Hahn et. al. , 2010), resulting in various health outcomes. BAC is a strong skin irritant at high concentrations (Basketter et. al. , 2004), whereas $CuSO_4$ can cause severe eye irritation (NPIC, 2012). Triclosan is a potential endocrine disruptor and detrimental to the immune functions and the central nervous system (Clayton et. al. , 2011). Thus, care must be taken when they are used in indoor environments.

Another concern lies in emergence of drug-resistant bacteria caused by the extensive use of biocides in indoor environments (Aiello and Larson, 2003, Levy, 2001). For instance, studies have been reported about emergence of *S. aureus* resistant to triclosan with a possibility of cross-resistance to other biocidal chemicals (Aiello and Larson, 2003, Suller and Russell, 2000). The resistance to BAC was also found in *Pseudomonas*, *S. aureus*, and *Listeria monocytogenes* (Loughlin et. al. , 2002, To et. al. , 2002).

Another potential side effect of the use of biocides is changes in indoor microbial communities and diversities. Studies indicate microbial communities in soil altered in response to biocide exposures (Hu et. al. , 2014, Zeng et. al. , 2011). Low microbial communities and diversities in house dust are associated with increased childhood asthma development (Maier et. al., 2010, Konya et. al., 2014, Ege et. al., 2011, Dannemiller et. al., 2014). Also, according to the Hygiene hypothesis, a lack of early childhood exposure to infectious agents, symbiotic microorganisms, and parasites increases susceptibility to allergic diseases by suppressing the natural development of the immune system (Brooks et. al. , 2013). Thus, care must be taken when biocides are applied indoors as they may create selective pressure for drug-resistant strains and changes in indoor microbial communities potentially associated with human health.

1.5. Objective of this study

House dusts have been used as surrogates of human exposures to indoor microbial materials (Wouters et. al., 2000, Frankel et. al., 2012, Wu et. al., 2012, Johansson et. al. , 2013, Douwes et. al., 2006) along with traditional growth-based methods to quantify culturable bacterial counts (Korthals et. al., 2008, Torvinen et. al., 2010) or DNA sequence-based methods to characterize bacterial communities and diversities (Rintala et. al., 2008). In this Master's research, I examined indoor floor dust-borne bacterial communities and diversities in relation to applications of three types of biocidal chemicals of BAC, $CuSO_4$, and triclosan. It is important to characterize changes in bacterial communities and diversities in response to biocidal chemical exposures. Specific aims of this research are to: (i) compare cultured and uncultured house dust-borne bacterial communities and diversities, (ii) quantify reduction of culturable house dust-borne bacterial counts in response to biocide exposures, and (iii) characterize change in bacterial communities and diversities in response to exposures to biocide exposures. To these ends, I used the conventional growth-based method to quantify changes in culturable bacterial counts and the next-generation sequencing (NGS) to analyze bacterial communities and diversities. The results obtained by this study can provide important insights into how the use of biocides in indoor environments can affect bacterial populations, communities, and diversities that are potentially important for human health.

2. Materials and Methods

2.1. Collection of house dust

The house dusts were collected to the filters from floors of washing rooms, kitchens, bedrooms, and laundry rooms in the student dormitory using a vacuum cleaner for 18 times. After sieving and weighing, the house dusts were suspended in the sterile buffer (deionized water with 42.5 mg/L, KH_2PO_4 (Sigma-Aldrich, St. Louis, MO, USA), 250 mg/L $MgSO_4 \cdot 7H_2O$ (Sigma-Aldrich, St. Louis, MO, USA), 8 mg/L NaOH (Sigma-Aldrich, St. Louis, MO, USA) and 0.02% Tween 80 (Sigma-Aldrich, St. Louis, MO, USA) (Karkkainen et. al., 2010).

2.2. Preparation of test biocides

Copper (II) Sulfate ($CuSO_4$) (Sigma-Aldrich, St. Louis, MO, USA), triclosan (Sigma-Aldrich, St. Louis, MO, USA) and benzalkonium chloride (BAC) (Sigma-Aldrich, St. Louis, MO, USA) powder were dissolved in the sterile buffer to obtain the initial concentration of 0.25% (v/v) (0.0090 g/ml) (Rowley, 1998) , 0.3% (v/v) (0.0045 g/ml) (SCCP, 2009) and 0.1% (w/w) (0.0010 g/ml) (U.S. FDA) in the house dust suspension, respectively. And then each biocide solution was diluted 10^2 and 10^4 times for experimental use.

2.3. Exposure to the biocides

House dust suspension prepared in the first part was divided into 10 tubes (5ml for each tube) after shaking for 10 min. Three different concentrations of each biocide were added into 9 out of 10 tubes (the left one tube acted as the blank). Then these 10 tubes were shaken in an orbital shaker for another 50 min. After shaking, the house dust suspension in each of the biocide-contained tubes was divided into two tubes (1.5ml for each tube), following the washing step. The left 2 ml in each tube was saved at -80 ℃. In the washing step, firstly, tubes were centrifuged to obtain the sediment. Secondly, take out supernatant from one of the two tubes and washing by buffer for two times. Trypticase soy agar (TSA) supplemented with 4 μg/ml itraconazole (TSAI) plates and trypticase soy

broths supplemented with 4μg/ml itraconazole (TSBI) were prepared. Duplicate ten-fold series of the suspension (after washing) of each sample were made and spread on TSAI plates (also TSBI broth) not containing the biocides. Similarly, duplicate ten-fold series of the suspension (without washing) of each sample were made and spread on TSAI plates (also TSBI broth) containing the same concentration of each biocide. The plates and broth were incubated at 32 ℃ for 3 days and the CFU number on each plate were counted after incubation.

2.4. DNA extraction and quantitative PCR

Based on the results of plate counting method, broth samples with 1/10000 diluent BAC concentration, 1/100 diluent $CuSO_4$ and triclosan concentration exposures were used for following experiments. DNA extraction of house dust suspension and cultured broth samples were performed using the PowerMax Soil DNA Isolation Kit (Mobio Laboratory, Carlsbad, CA, USA) with the modification of adding 0.1mm diameter glass beads (300 mg) and 0.5mm diameter beads (100 mg) to the microcentrifuge tube (Hospodsky et. al. , 2012). The samples were first homogenized for 4 minutes by a bead beater (BioSpec. Inc.Bartlesville, OK, USA). And then proceed to the DNA extraction following the protocol. The samples were eluted with 50 ul 10 μM Tris buffer and keep at -20 ℃ before amplification.

All qPCR assays were performed on an ABI 7300 system (Applied Biosystems, Foster City, CA, USA). Forward primer 5'- TCCTACGGGAGGCAGCAGT-3', reverse primer 5'-GGACT ACCAGGGTATCTAATCCTGTT-3', and the TaqMan probe, (6-FAM)-5'-CGTATTACCGCG GCTGCTGGCAC-3'-(BHQ1) targeted the 331 to 797 E.coli numbering region of the 16S rDNA were used. For each assay, 50 μl qPCR mixtures were prepared, containing 25 μl of 2 × TaqMan Universal PCR Master Mix (Life Technologies), 1 μl of 10 μM probe, 1μl of each 10 μM primer, 20 μl of nuclease-free water and 2 μl of DNA template . Duplication of each sample, no-template controls, and positive controls were included in each run. Thermocycler conditions are 2 minutes at 95 ℃ for AmpErase uracil-N-glycosylase (UNG) incubation, 10 minutes at 95 ℃ for AmpliTaq Gold Activation and 45 subsequent cycles of 15 seconds at 95℃, 45 seconds at 56 ℃ and 90 seconds at 72

℃. Real-time PCR standard curves of genome copies versus cycle threshold number for bacteria were built using known amounts of *E.coli* (ATCC 25922) genomic DNA. To develop the standard curve, the *E.coli* genomic DNA was first amplified by the conventional PCR using the same primer. After amplification, the original genome copies number was calculated based on the genome mass and length. Six independent dilution series were produced corresponding to 10^1 to 10^6 genome copies to produce the standard curve.

2.5. Next-generation DNA sequencing processing and analyses

Library preparation was performed following the Illumina 16S Metagenomic Sequencing Library Preparation protocol. The 16S rRNA genes were amplified using the forward primer=5'TCGTCGGCAGCGTCAGATG TGTATAAGAGACAGCCTACGGGNGGCWG CAG and reverse primer = 5'GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGAC TACHVGGGTATCTAATCC. Each PCR reaction was 25µl, including 12.5µl of 2× KAPA HiFi HotStart ReadyMix, 5µl of each 1µM primer and 2.5µl of the DNA template. PCR was performed using the following condition: initial denaturation at 95℃ for 3min, 35 cycles of 95℃ dissociation for 30 seconds, annealing at 55℃ for 30 seconds and extension at 72℃ for 30 seconds, followed by final extension at 72℃ for 5 min. Then PCR clean-up was conducted using AMPure XP beads to purify the 16S V3 and V4 amplicon away from free primers and primer dimer species. Dual indices were attached by Index PCR following the thermal cycler conditions: 95℃ for 3 min, 8 cycles of 95℃ for 30 seconds, 55℃ for 30 seconds and 72℃ for 30 seconds, followed by 72℃ for 5 min. The second PCR clean-up was conducted after Index PCR.

Library quantification was performed using PicoGreen method. The quantified libraries were pooled and denatured with NaOH, diluted with hybridization buffer before Miseq sequencing. 30% PhiX were added to serve as an internal control for these low-diversity libraries. Libraries were then loaded onto a MiSeq reagent cartridge and then onto the instrument. Then the 16S rDNA next generation sequencing was performed by using MiSeq sequencing system, which relies on the

fluorescence generated by the incorporation of fluorescently labeled nucleotides into the growing strand of DNA (Quail et. al. , 2012).

After samples are loaded, the Miseq system provides secondary analysis using the Miseq Reporter Software (MSR). Sequence reads were trimmed of primer sequences and multiplexing barcodes. Truncation of sequence reads not having an average quality score of 20 was also completed. Further sequence reads processing was performed using QIIME (version 1.9.0) (Caporaso et. al. , 2010). QIIME quality trimming was performed following criteria: 1) no ambiguous base calls; and 2) minimum sequence length of 200bp after trimming. Taxonomic ranks were assigned to each sequence using Ribosomal Database project (RDP) Naïve Bayesian Classifier (Wang et. al. , 2007), using 0.8 confidence values as cutoff.

The bacterial community richness index, community diversity index, data processing and phylogeny-based analysis were performed using Mothur (Schloss et. al. , 2009). Chao 1 richness estimators, ACE, Shannon and Simpson diversity index were calculated. Chao 1 richness estimator showed the estimated number of different species represented in a given sample and ACE (abundance-based coverage) also incorporates the data from all species with fewer than 10 individuals. Simpson and Shannon were community diversity indices. Simpson indicated the probability that two individuals randomly selected from a sample will belong to the same species, and the higher the number, the lower the diversity. Shannon quantified the uncertainty, indicating higher diversity with higher number. The equations to calculate the Shannon and Simpson indices are:

$$\text{Shannon Index (H)} = -\sum_{i=1}^{s} p_i \ln p_i$$

$$\text{Simpson Index (D)} = \frac{1}{\sum_{i=1}^{s} p_i^{\,2}}$$

In the Shannon index, *p* is the proportion (n/N) of individuals of one particular species found (n) divided by the total number of individuals found (N), ln is the natural log; Σ is the sum of the calculations, and *s* id the number of species. In the Simpson index, *p* is the proportion (n/N) of

individuals of one particular species found (n) divided by the total number of individuals found (N), Σ is still the sum of the calculations, and *s* is the number of species.

Weighted Unifrac calculations were performed to assess differences among samples based on phylogenic information (Lozupone and Knight, 2005). The histogram was created using Microsoft Excel 2010 (Microsoft, Redmond, Washington, USA).

The whole experiment process is showing in Figure 1.

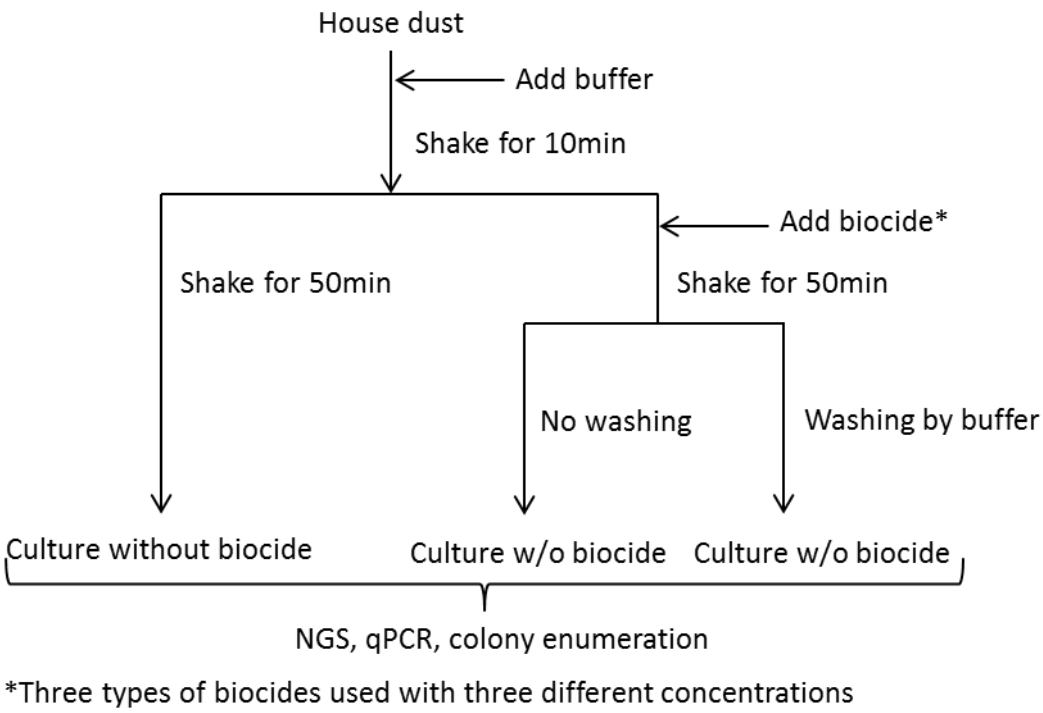


Figure 1. Flow chart of experimental process

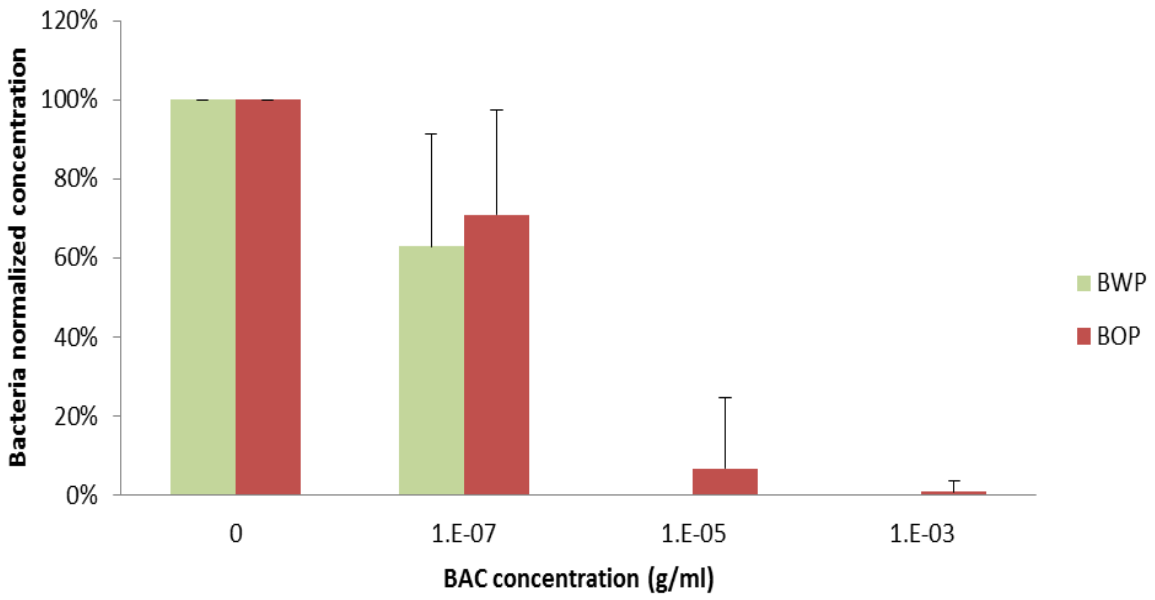
3. Results

3.1. Plate counting results

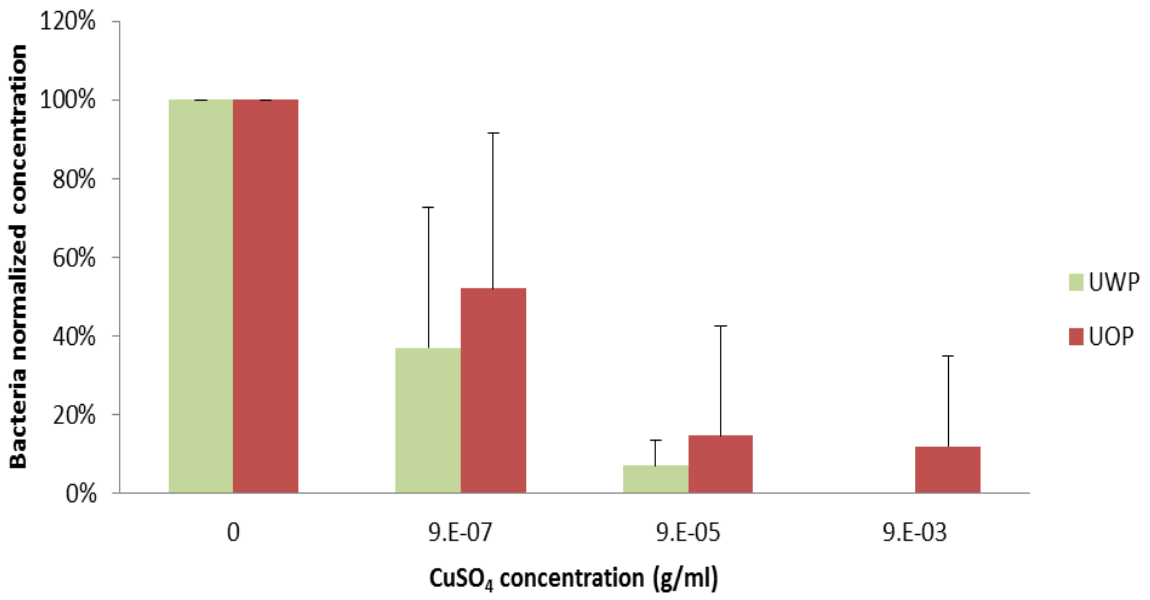
The CFU number on each plate was counted to obtain the counts of culturable house dust-borne bacteria on floors of the student dormitory (Table 1). After data normalization, the average bacterial concentration of each group can be obtained (Figure 2). Based on different experimental treatment, the samples can be divided into three groups including blank group (no biocide exposure and cultured without biocide), XOP group ((with biocide exposure and cultured without biocide, X=B (BAC), T (triclosan), or U ($CuSO_4$)) and XWP group (with biocide exposure and cultured with biocide). The bacterial counts of XOP group and XWP group were compared with blank group to evaluate the three biocides efficacy. It's obvious that either for XOP group or XWP group, bacterial counts increased with the decrease of biocide concentration. Results also showed that XWP group has smaller CFU number compared to the XOP group. The bacterial counts reduction ratios of XOP and XWP groups, compared with blank group, were calculated in Table 2. For different concentrations of three biocides, the reduction ratios of XWP group were always higher than (or equal to) that of XOP group. For instance, the highest concentration of three biocides could reach to the 100% reduction ratios for XWP group, whereas for XOP group, the reduction ratios of BOP (99%) and UOP (88%) were lower than 100%.

Table 1. Bacteria colony forming units (CFU) detected on each nutrient agar plate (CFU/100 µl). BWP: samples with BAC exposure and plate-cultured with BAC; BOP: samples with BAC exposure and plate-cultured without BAC; UWP: samples with $CuSO_4$ exposure and plate-cultured with $CuSO_4$; UOP: samples with $CuSO_4$ exposure and plate-cultured without $CuSO_4$; TWP: samples with triclosan exposure and plate-cultured with triclosan; TOP: samples with triclosan exposure and plate-cultured without triclosan; Blank: no biocide exposure and cultured without biocide.

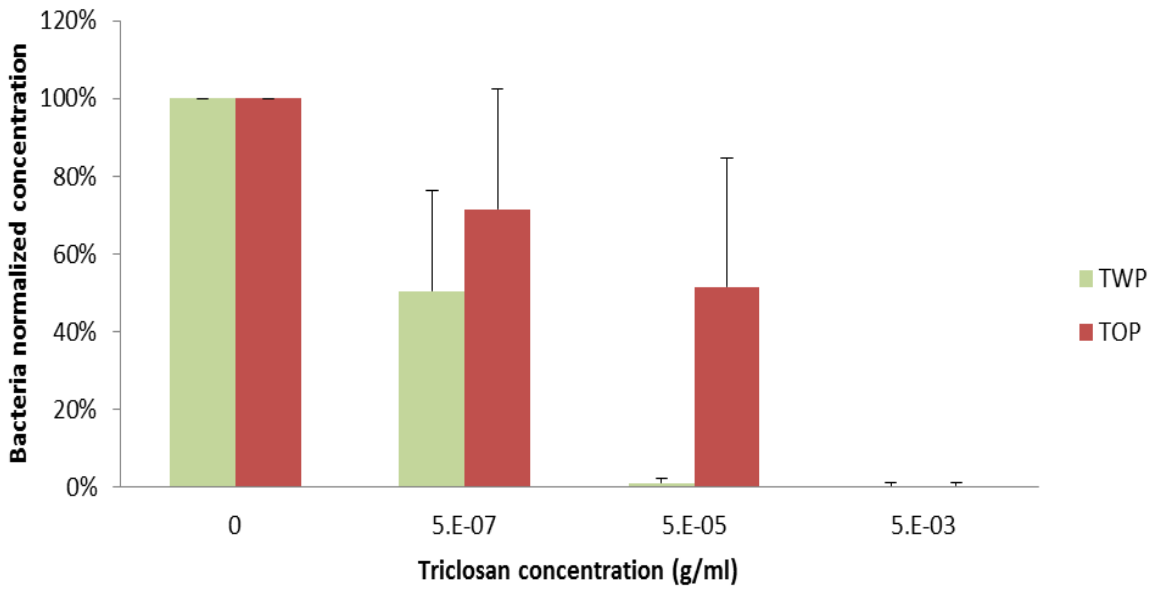
Sampling site	ID	No exposure	BAC exposure (g/ml)						$CuSO_4$ exposure (g/ml)						Triclosan exposure (g/ml)					
		Blank	1.E-03		1.E-05		1.E-07		9.E-03		9.E-05		9.E-07		5.E-03		5.E-05		5.E-07	
			BWP	BOP	BWP	BOP	BWP	BOP	UWP	UOP	UWP	UOP	UWP	UOP	TWP	TOP	TWP	TOP	TWP	TOP
Washing room	H9	900	0	0	0	1	410	1120	0	1	0	1	36	22	0	20	0	160	390	280
	H13	605	0	0	0	4	520	980	0	3	40	76	250	400	0	1	0	460	300	560
	H16	955	0	0	0	22	430	570	0	15	52	64	360	173	0	0	16	430	290	N/A
Bedroom	H10	125	0	0	0	6	80	70	0	2	13	20	21	320	0	N/A	0	101	123	N/A
	H14	250	0	0	0	7	89	74	0	2	15	14	42	39	0	0	0	22	30	120
	H17	885	0	0	0	32	590	460	0	0	180	131	500	400	0	0	0	153	480	610
Kitchen	H11	325	0	1	0	5	268	170	0	226	4	13	10	320	13	N/A	N/A	N/A	N/A	100
	H15	200	0	14	0	100	300	330	0	58	25	160	290	250	1	1	6	190	62	200
	H18	835	0	0	0	20	800	850	0	23	62	59	770	540	0	1	2	650	670	1010
Laundry room	H12	235	0	2	0	1	32	140	0	22	0	1	12	20	0	0	1	96	128	310


120%
100%
80%
60%
40%
20%
0%
Bacteria normalized concentration
0
1.E-07
1.E-05
1.E-03
BAC concentration (g/ml)
BWP
BOP

(A)


120%
100%
80%
60%
40%
20%
0%
Bacteria normalized concentration
0
9.E-07
9.E-05
9.E-03
CuSO4 concentration (g/ml)
UWP
UOP

(B)



(C)

Figure 2. Average bacterial concentrations of different samples. (A) Bacterial concentrations of samples exposed to different BAC concentrations; (B) Bacterial concentrations of samples exposed to different $CuSO_4$ concentrations; (C) Bacterial concentrations of samples exposed to different triclosan concentrations. BWP: samples with BAC exposure and plate-cultured with BAC; BOP: samples with BAC exposure and plate-cultured without BAC; UWP: samples with $CuSO_4$ exposure and plate-cultured with $CuSO_4$; UOP: samples with $CuSO_4$ exposure and plate-cultured without $CuSO_4$; TWP: samples with triclosan exposure and plate-cultured with triclosan; TOP: samples with triclosan exposure and plate-cultured without triclosan.

Table 2. Bacterial counts reduction ratios of different groups compared with blank group. (A) Bacterial counts reduction ratios of BWP and BOP groups compared with blank group; (B) Bacterial counts reduction ratios of TWP and TOP groups compared with blank group; (C) Bacterial counts reduction ratios of UWP and UOP groups compared with blank group. BWP: samples with BAC exposure and plate-cultured with BAC; BOP: samples with BAC exposure and plate-cultured without BAC; UWP: samples with $CuSO_4$ exposure and plate-cultured with $CuSO_4$; UOP: samples with $CuSO_4$ exposure and plate-cultured without $CuSO_4$; TWP: samples with triclosan exposure and plate-cultured with triclosan; TOP: samples with triclosan exposure and plate-cultured without triclosan.

(A)

BAC Concentration (w/w)	BWP	BOP
0.1%	100%	99%
0.001%	100%	93%
0.00001%	37%	29%

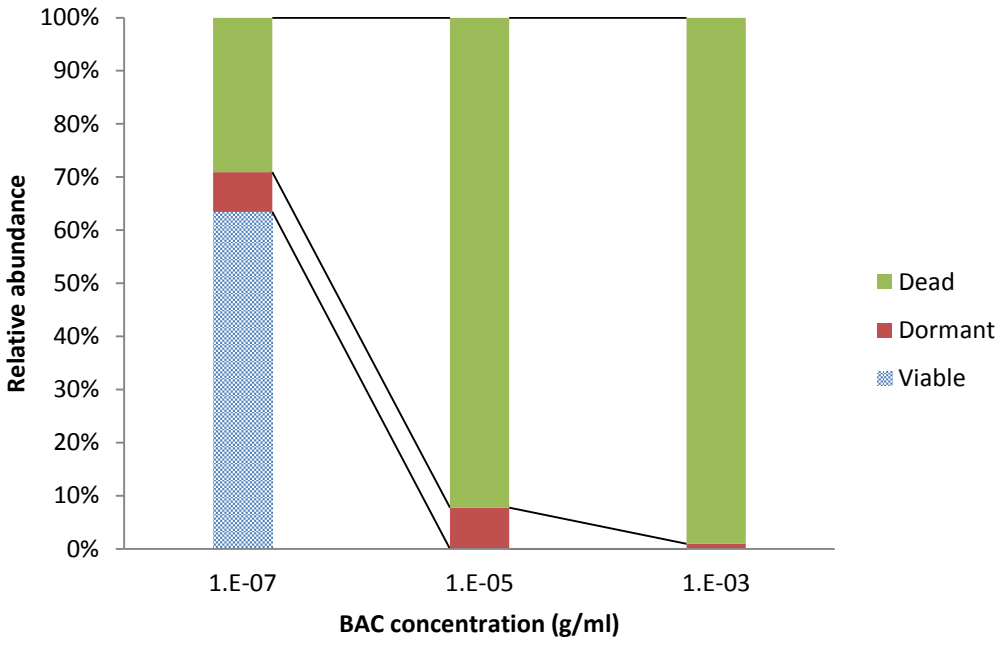
(B)

Triclosan Concentration (v/v)	TWP	TOP
0.3%	100%	100%
0.003%	99%	48%
0.00003%	50%	29%

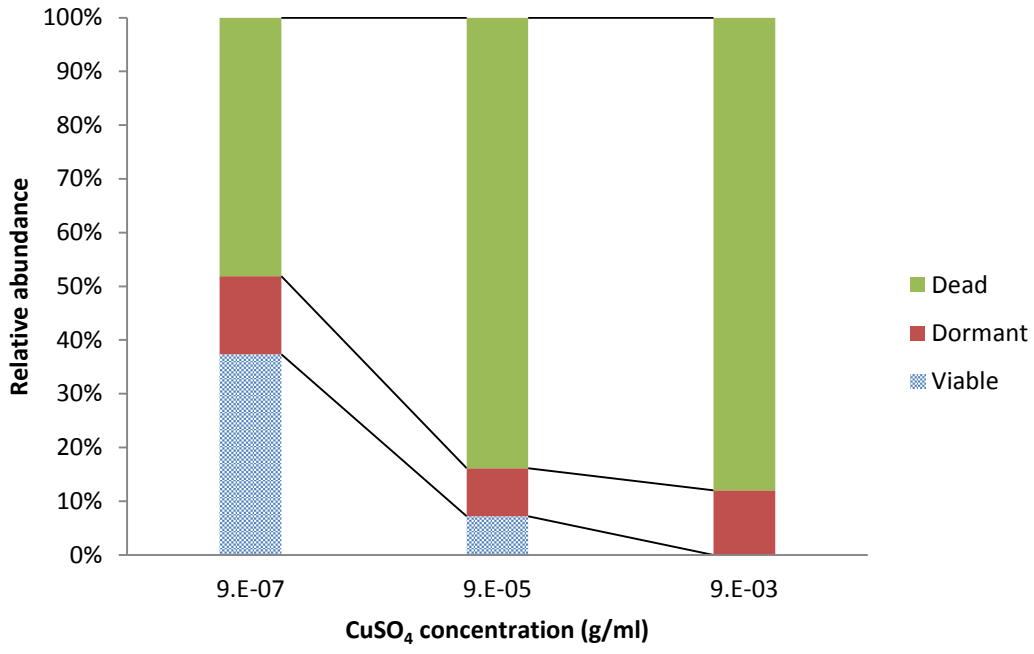
(C)

$CuSO_4$ Concentration (v/v)	UWP	UOP
0.25%	100%	88%
0.0025%	93%	75%
0.000025%	63%	48%

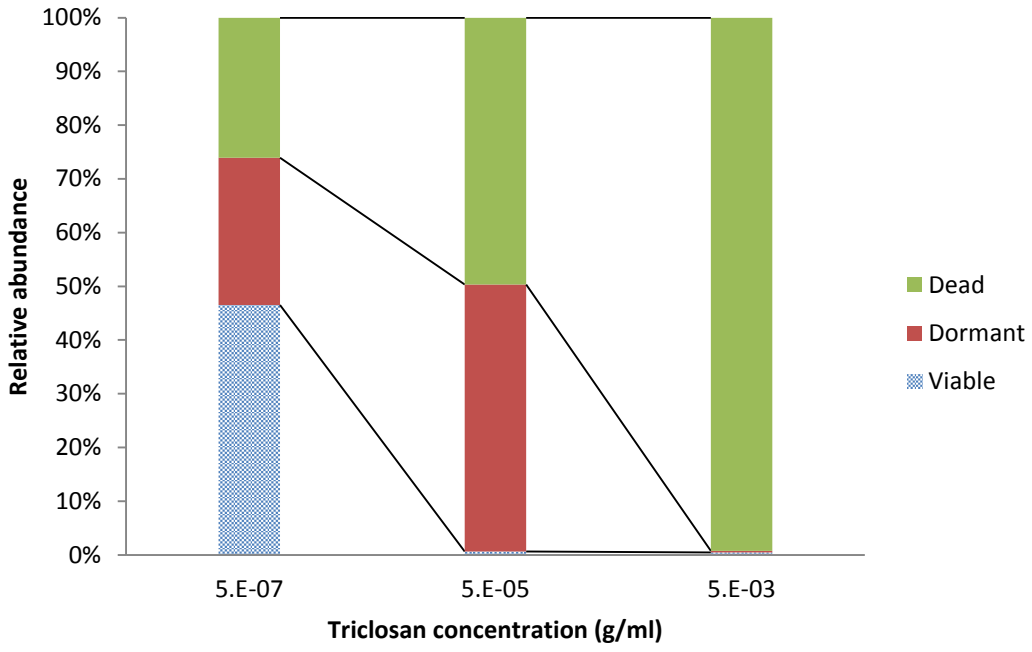
The bacteria exposed to the biocides were categorized into either viable, dormant or dead groups according to their definitions, using the data of blank, XWP and XOP groups, showing in Figure 3. For samples with the lowest concentrations of BAC and triclosan exposures, the viable and dormant bacteria were the main component (70.91% for BAC and 81.05% for triclosan), whereas for samples with the lowest concentration $CuSO_4$ exposure, dead bacteria occupied almost half (48.09%) of the total bacteria. For samples having the highest biocide concentrations exposure, which are also the recommended application concentrations of three biocides, most of the bacteria were killed (99.06% for BAC, 99.56% for Triclosan, 87.97% for $CuSO_4$), rather than dormant or viable. The second higher concentrations of biocide BAC and $CuSO_4$ can also kill 93.13% and 85.27% of total bacteria. However, around half of the bacteria were inhibited rather than killed by applying the second higher concentration of triclosan.


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Relative abundance
1.E-07
1.E-05
1.E-03
BAC concentration (g/ml)
Dead
Dormant
Viable

(A)


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Relative abundance
9.E-07
9.E-05
9.E-03
CuSO4 concentration (g/ml)
Dead
Dormant
Viable

(B)



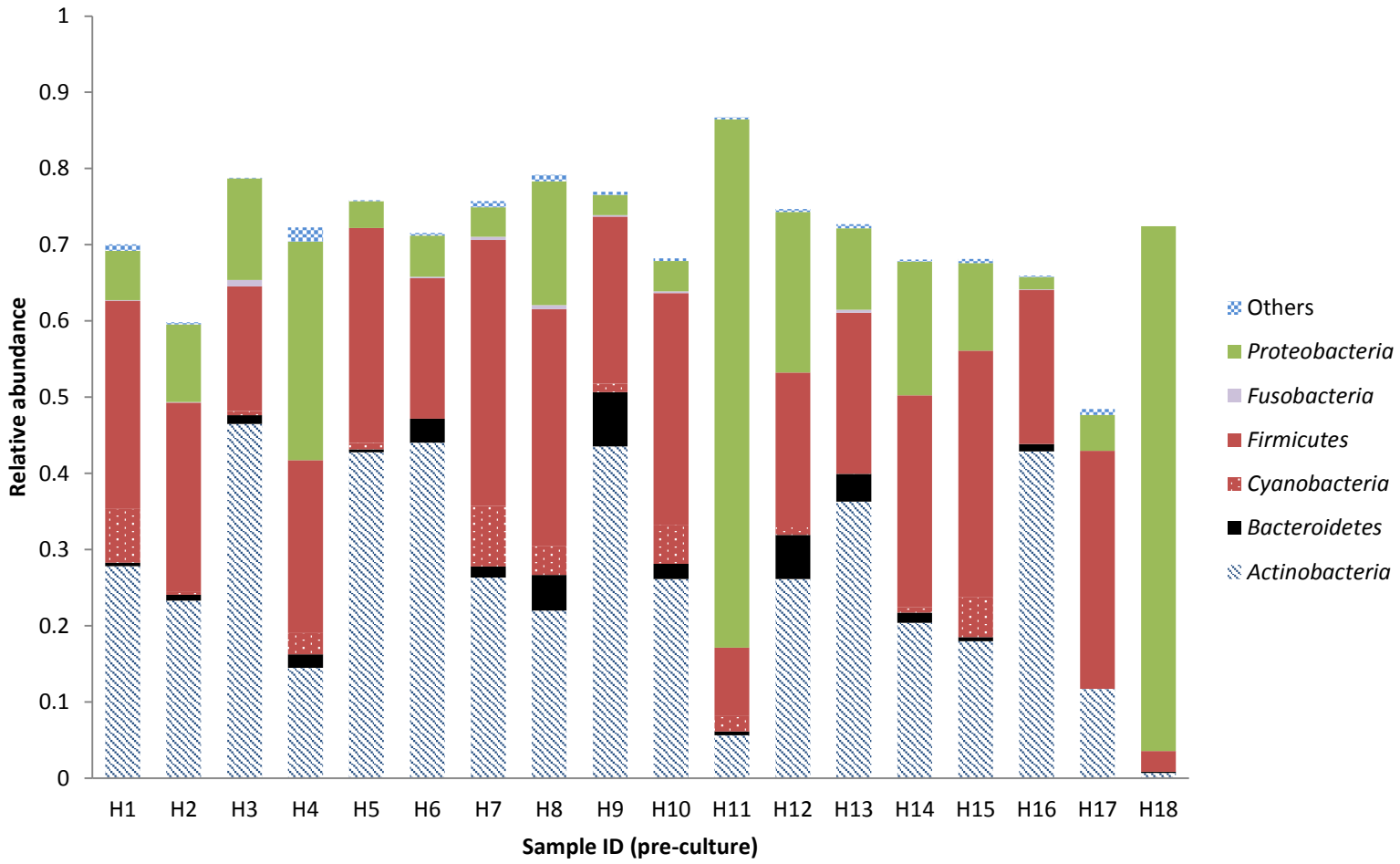
(C)

Figure 3. Bacterial vital status (viable, dormant, and dead) composition after exposing to three different biocides. (A) Bacterial vital status after exposing to different concentrations of BAC; (B) Bacterial vital status after exposing to different concentrations of $CuSO_4$; (C) Bacterial vital status after exposing to different concentrations of triclosan.

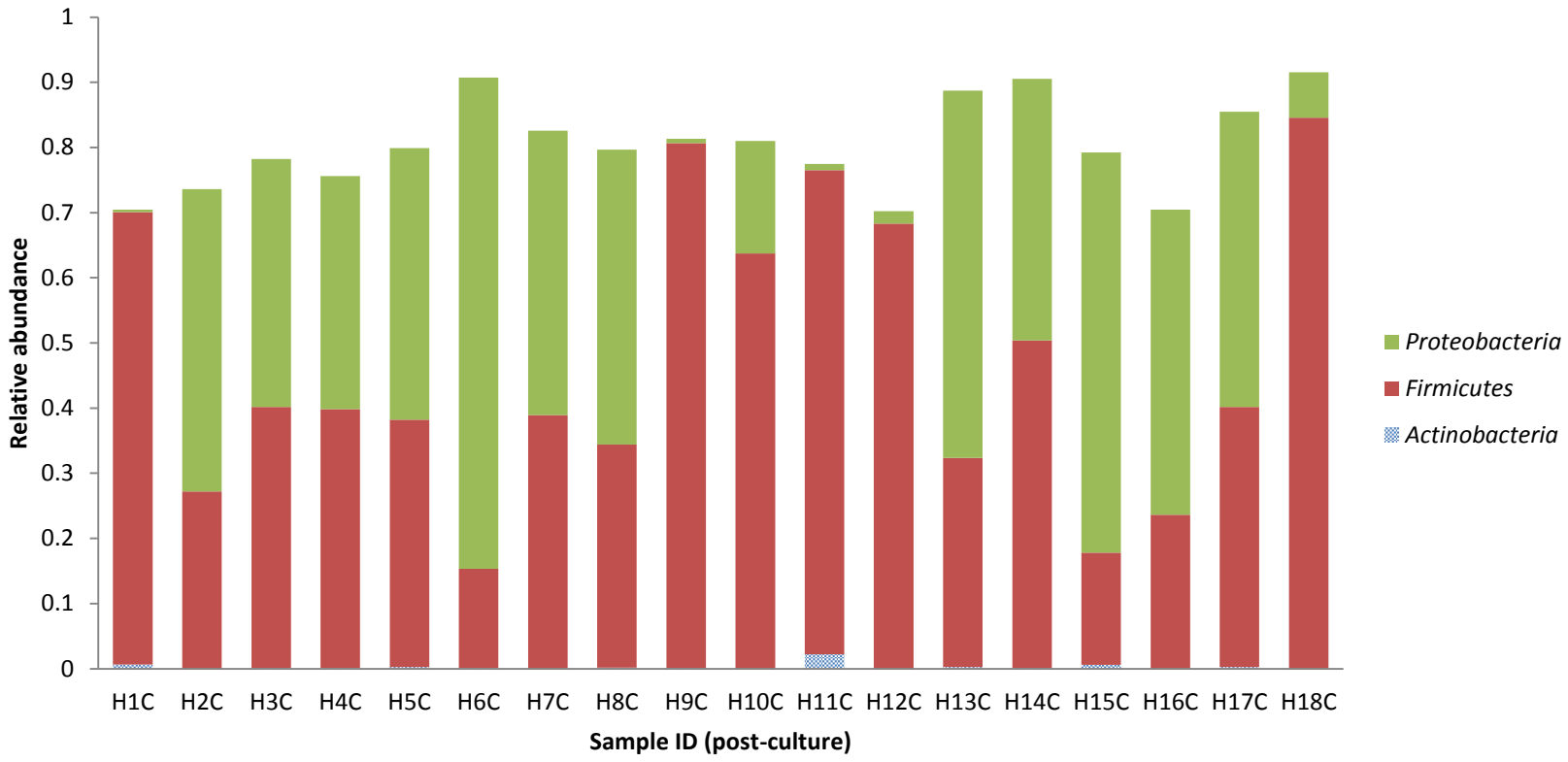
3.2. Bacterial composition results

Bacterial composition results were obtained from NGS data. According to different experimental treatment, the samples were characterized into four different groups including pre-culture samples (no biocide exposure and without cultivation) with the name HN (N=sampling number), post-culture samples (no biocide exposure and with cultivation) with the name HNC (N=sampling number), XO samples ((with biocide exposure and cultured without biocide, X=B (BAC), U ($CuSO_4$), T (triclosan)), and XW samples (with biocide exposure and cultured with biocide). Classified sequences belonged to 18 phyla among all samples were observed and included three main phyla: *Actinobacteria*, *Firmicutes*, and *Proteobacteria*. The bacterial community compositions of pre-culture and post-culture samples were compared (Figure 4). The overall bacterial composition of different pre-culture samples was similar, while the distribution of each phylum varied. The same thing happened on post-culture samples. And the most three abundant phyla accounted for 92.9±6.9% among all pre-culture samples, but 100% for all post-culture samples. For post-culture samples, *Firmicutes* and *Proteobacteria* phyla were dominated, which accounted more than 97.1% among all samples. On a genus level, all 312 different genera were detected among all samples. The most abundant five genera were *Atopobium*, *Corynebacterium*, *Gardnerella*, *Lactobacillus*, and *Staphylococcus*.

Bacterial community compositions for samples exposed to three different biocides were also studied (Figure 5). The phyla of *Actinobacteria*, *Firmicutes*, and *Proteobacteria* were still three main phyla among all samples. In all, the bacterial compositions were not very similar and the distribution of each phylum was also different. For samples having biocide BAC exposure, the phylum *Firmicutes* had a similar distribution between post-cultured samples and BO samples, whereas for BW samples, *Firmicutes* distribution decreased among most of the samples (70%). For biocide triclosan, the *Firmicutes* distributions increased in TO samples but decreased in TW samples, owing to a decreased distribution of phylum *Proteobacteria* in TO samples and increased distribution in TW samples. The phylum *Firmicutes*'s relative abundance kept increasing in UO samples and also increased in70% of the UW samples.

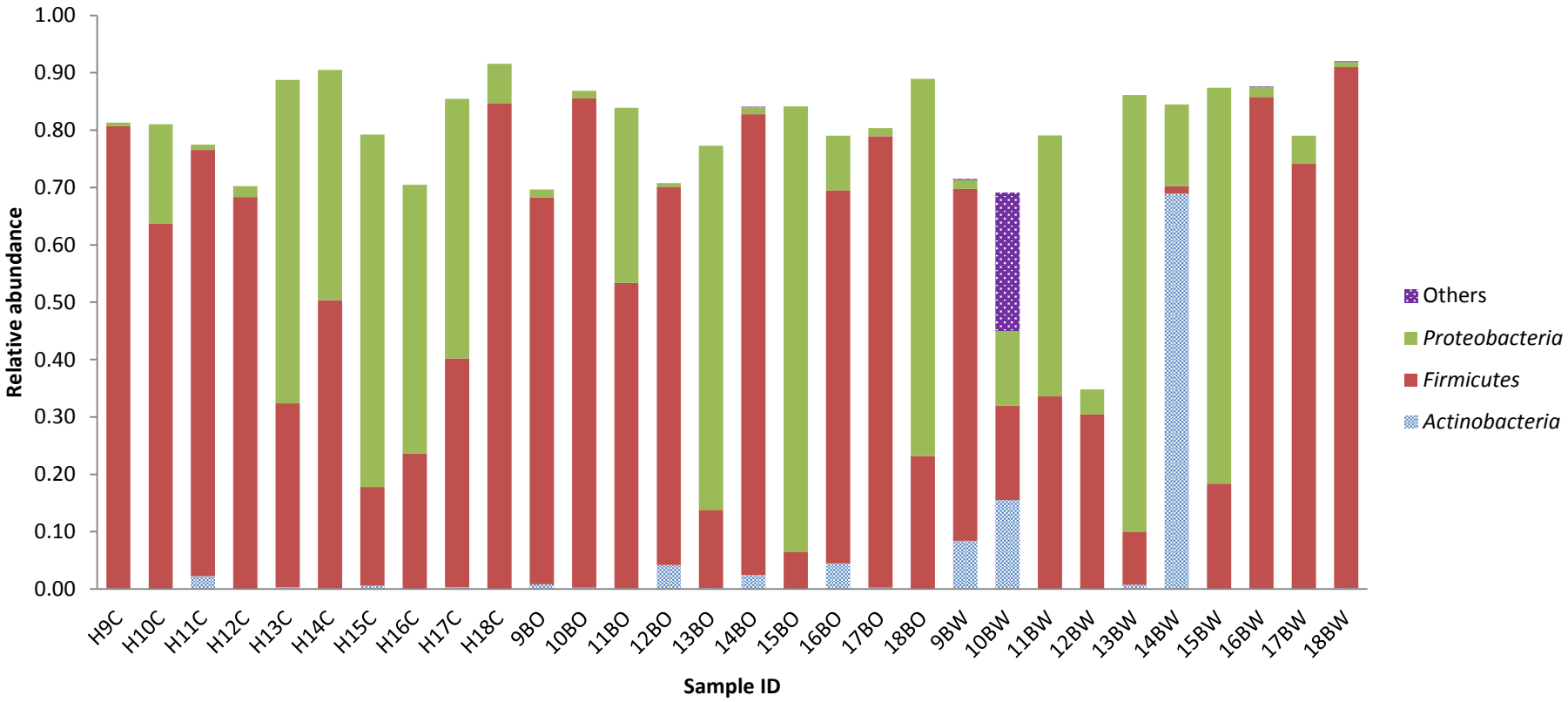

1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Relative abundance
H1
H2
H3
H4
H5
H6
H7
H8
H9
H10
H11
H12
H13
H14
H15
H16
H17
H18
Sample ID (pre-culture)
Others
Proteobacteria
Fusobacteria
Firmicutes
Cyanobacteria
Bacteroidetes
Actinobacteria

(A)

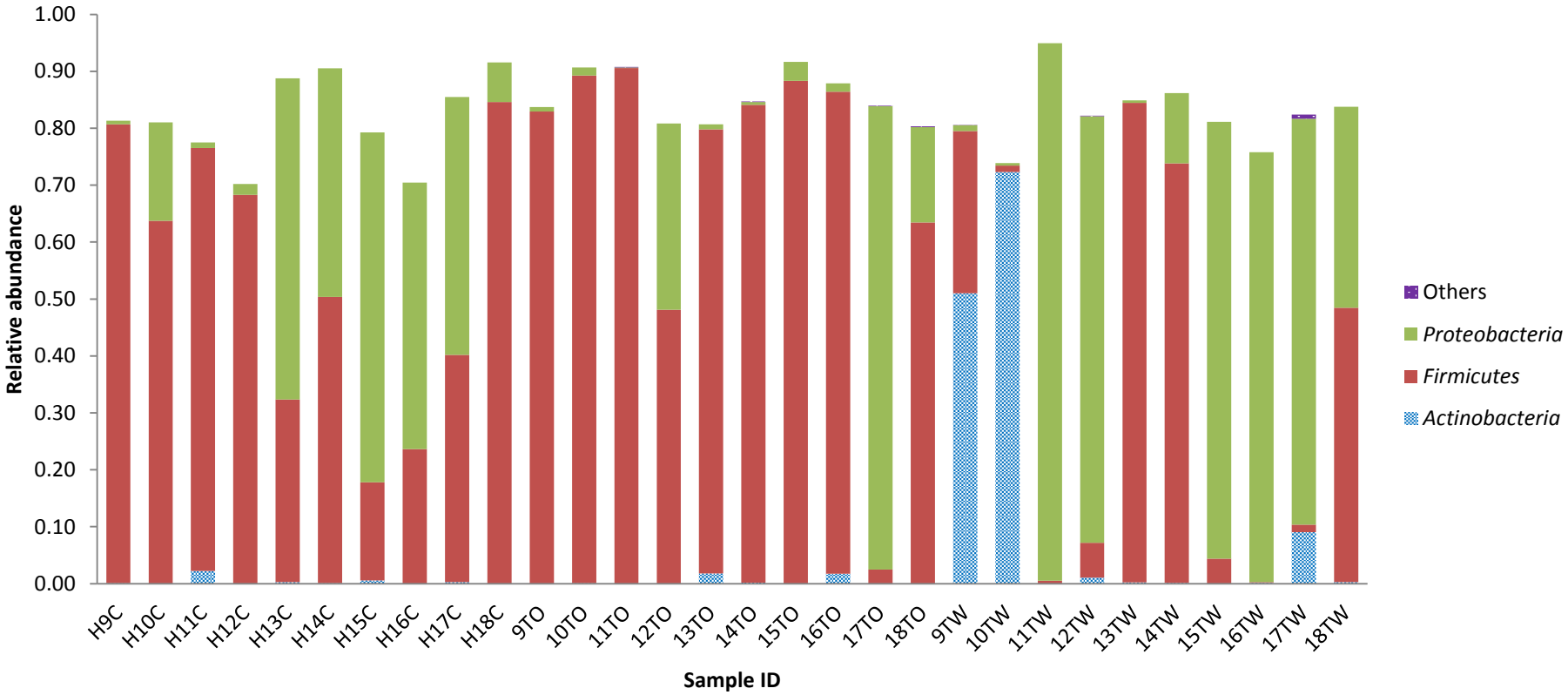


(B)

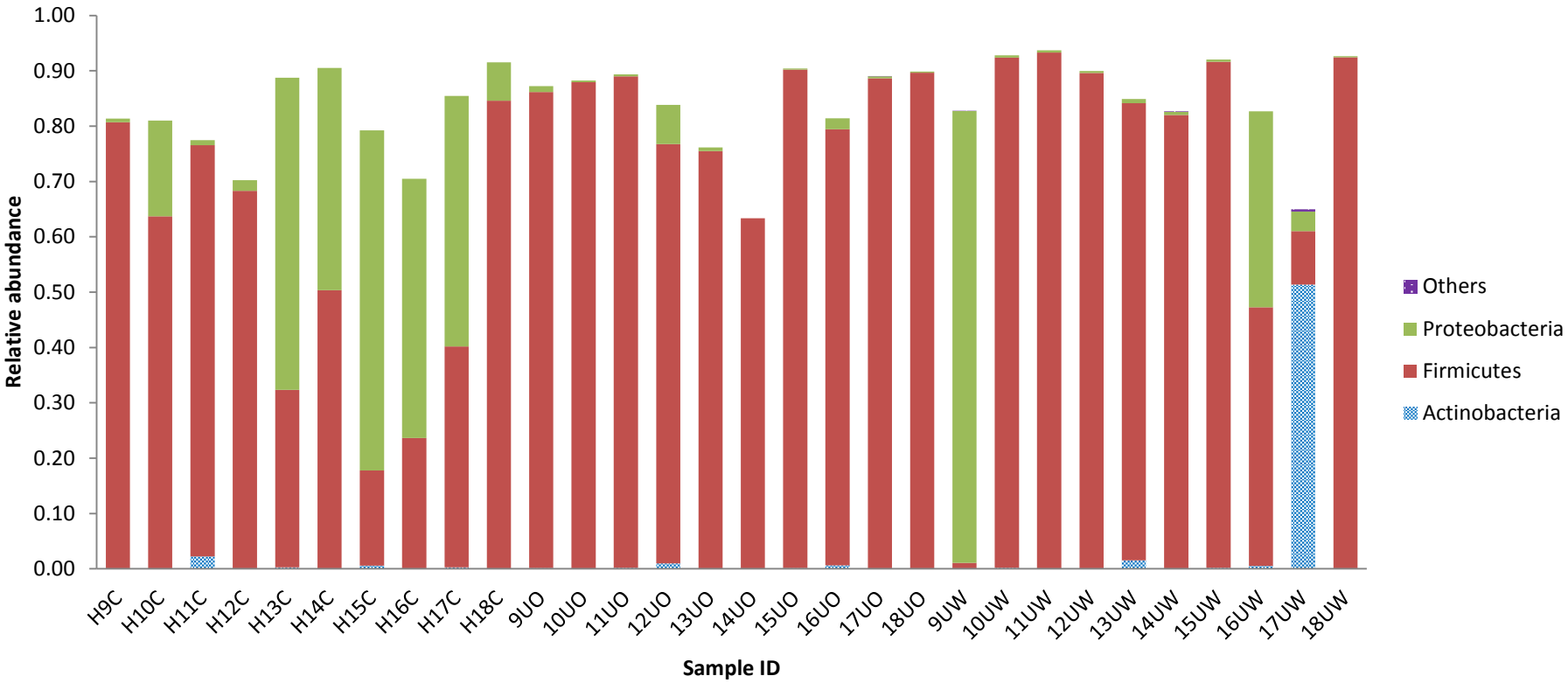
Figure 4. Bacterial community composition of pre-culture and post-culture samples. (A) Bacterial community composition of pre-culture samples; (B) Bacterial community composition of post-culture samples. Pre-culture: samples without biocide exposure and cultivation; Post-culture: samples without biocide exposure and with cultivation. The y-axis shows the sequences percentage which has be identified.


1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Relative abundance
H9C
H10C
H11C
H12C
H13C
H14C
H15C
H16C
H17C
H18C
9BO
10BO
11BO
12BO
13BO
14BO
15BO
16BO
17BO
18BO
9BW
10BW
11BW
12BW
13BW
14BW
15BW
16BW
17BW
18BW
Sample ID
Others
Proteobacteria
Firmicutes
Actinobacteria

(A)


1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Relative abundance
H9C
H10C
H11C
H12C
H13C
H14C
H15C
H16C
H17C
H18C
9TO
10TO
11TO
12TO
13TO
14TO
15TO
16TO
17TO
18TO
9TW
10TW
11TW
12TW
13TW
14TW
15TW
16TW
17TW
18TW
Sample ID
Others
Proteobacteria
Firmicutes
Actinobacteria

(B)



(C)

Figure 5. Bacterial compositions of samples with different biocides exposures. (A) Bacterial composition of BO and BW samples; (B) Bacterial composition of UW and UO samples; (C) Bacterial composition of TW and TO sample. BW: samples with BAC exposure and cultured with BAC; BO: samples with BAC exposure and cultured without BAC; UW: samples with $CuSO_4$ exposure and cultured with $CuSO_4$; UO: samples with $CuSO_4$ exposure and cultured without $CuSO_4$; TW: samples with triclosan exposure and cultured with triclosan; TO: samples with triclosan exposure and cultured without triclosan. The y-axis shows the sequences percentage which has be identified.

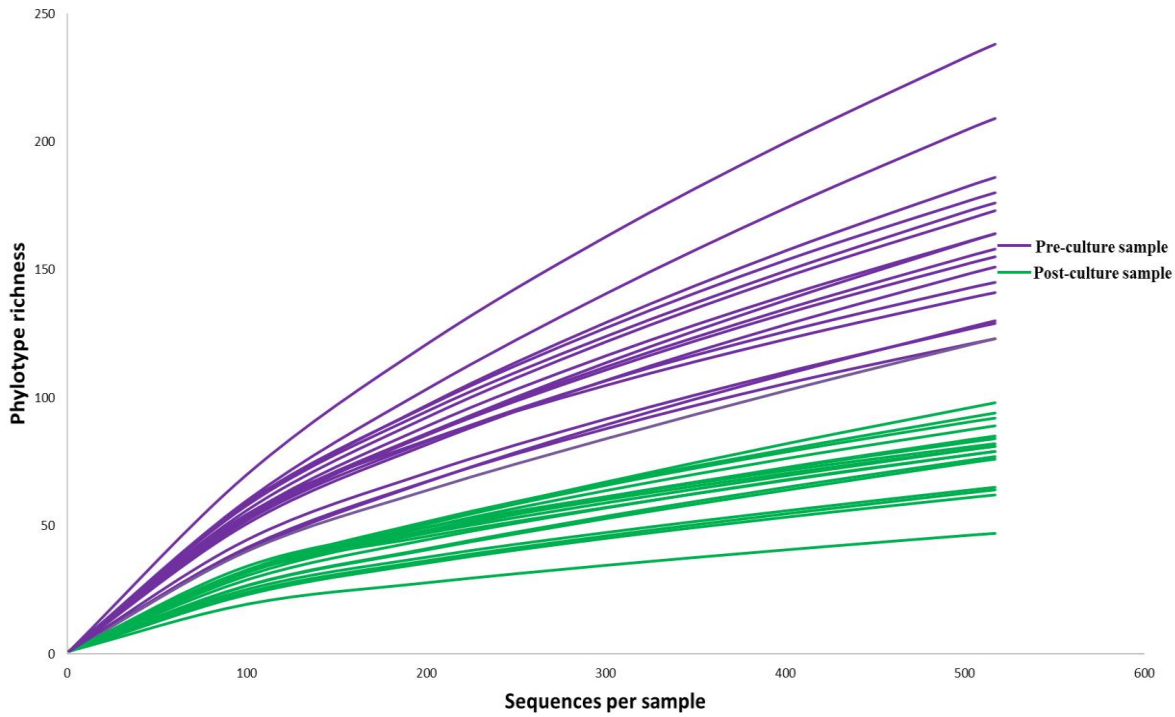
3.3. Bacteria diversity within individual samples

To further estimate the diversity and richness of different samples, the Mothur software was used to calculate the Chao1 estimator, ACE, Shannon, and Simpson diversity indexes (Table 3). The rarefaction curves of four groups were then plotted with line chart in Microsoft Excel (Figure 6), indicating that 517 reads per sample (the minimum number of sequences passing all quality control measures across the samples) for the final analysis was adequate since increasing the number of reads beyond that value had minimal impact on the number of OTUs. A total of 8011 operational taxonomic units (OTUs) based on 97% sequence similarity were observed. Measurements of alpha-diversity indicated that the bacterial community diversity of pre-culture samples (2745 OTUs observed) was significantly higher than post-culture samples (1335 OTUs observed). In addition, the alpha diversity comparison among post-culture samples, XO samples and XW samples were also performed (Figure 6). For XO groups, the observed OTUs in TO and UO samples were reduced with the reduction ratios of 26% and 24%, compared with post-culture samples. While for BO samples, the observed OTUs were 1% larger than post-culture samples. For TW and UW samples, similar with TO and UO samples, the observed OTUs were 1% and 29% lower than the post-culture samples. The observed OTUs in BW samples were 3% higher than the post-culture samples. However, it’s evident that post-culture, XO and XW samples were all lower than pre-culture samples based on observed OTUs.

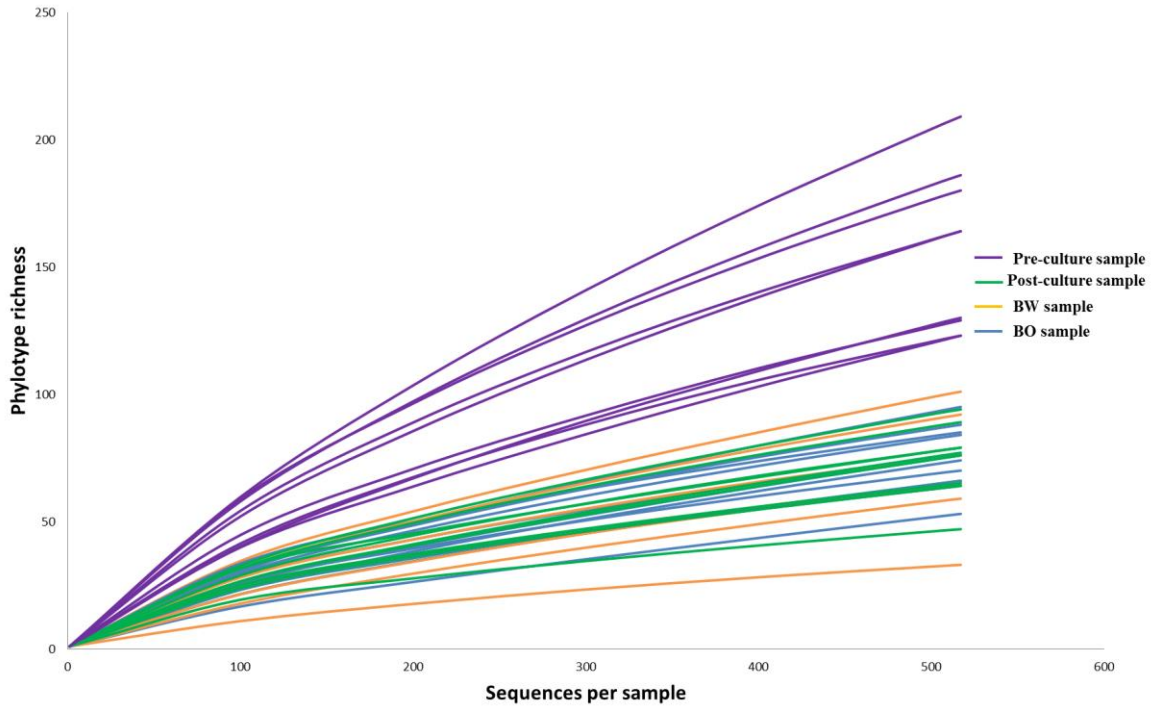
Table 3. Diversity indices for all samples, including pre-culture samples, post-culture samples, XO samples and XW samples. XO: samples with biocide X exposure and cultured without biocide X; XW: samples with biocide X exposure and cultured with biocide X.

Sample ID	Sequences	Phylotypes	Chao 1	Simpson	Shannon	ACE
10BO	517	53	258	0.18	2.18	915
11BO	517	88	162	0.12	2.98	358
11BW	517	101	329	0.08	3.30	681
12BO	517	74	216	0.21	2.33	337
13BO	517	84	166	0.13	2.83	346.
13BW	517	66	336	0.20	2.27	313
14BO	517	76	180	0.10	2.94	312
14BW	517	76	141	0.09	2.95	265
15BO	517	70	166	0.11	2.85	163
15BW	517	66	201	0.14	2.61	432
16BO	517	85	175	0.08	3.25	291
16BW	517	33	60	0.42	1.35	60
17BO	517	76	306	0.17	2.57	566
17BW	517	65	178	0.24	2.17	161
18BO	517	64	220	0.13	2.72	355
18BW	517	59	188	0.32	1.81	407
9BO	517	95	401	0.16	2.80	487
9BW	517	92	206	0.20	2.81	321
10UO	517	55	129	0.29	1.89	243
10UW	517	52	158	0.24	2.06	214
11UO	517	56	116	0.20	2.19	197
11UW	517	40	72	0.27	1.88	123
12UO	517	72	185	0.11	2.75	193
12UW	517	45	95	0.25	2.00	148
13UO	517	63	137	0.23	2.46	122
13UW	517	65	139	0.15	2.63	222
14UW	517	55	149	0.19	2.25	121
15UO	517	47	97	0.21	2.11	139
15UW	517	56	135	0.21	2.19	234
16UO	517	77	195	0.33	2.24	283
16UW	517	93	191	0.13	3.15	275
17UO	517	53	84	0.20	2.19	95
17UW	517	124	332	0.11	3.34	587
18UO	517	45	103	0.30	1.77	222
18UW	517	36	71	0.32	1.64	105
9UO	517	71	161	0.13	2.68	284
9UW	517	69	177	0.18	2.48	228
10TO	517	44	94	0.22	2.10	147
10TW	517	90	426	0.20	2.71	731.
11TO	517	45	151	0.25	1.84	417
11TW	517	40	82	0.41	1.60	102
12TO	517	80	246	0.11	2.85	264

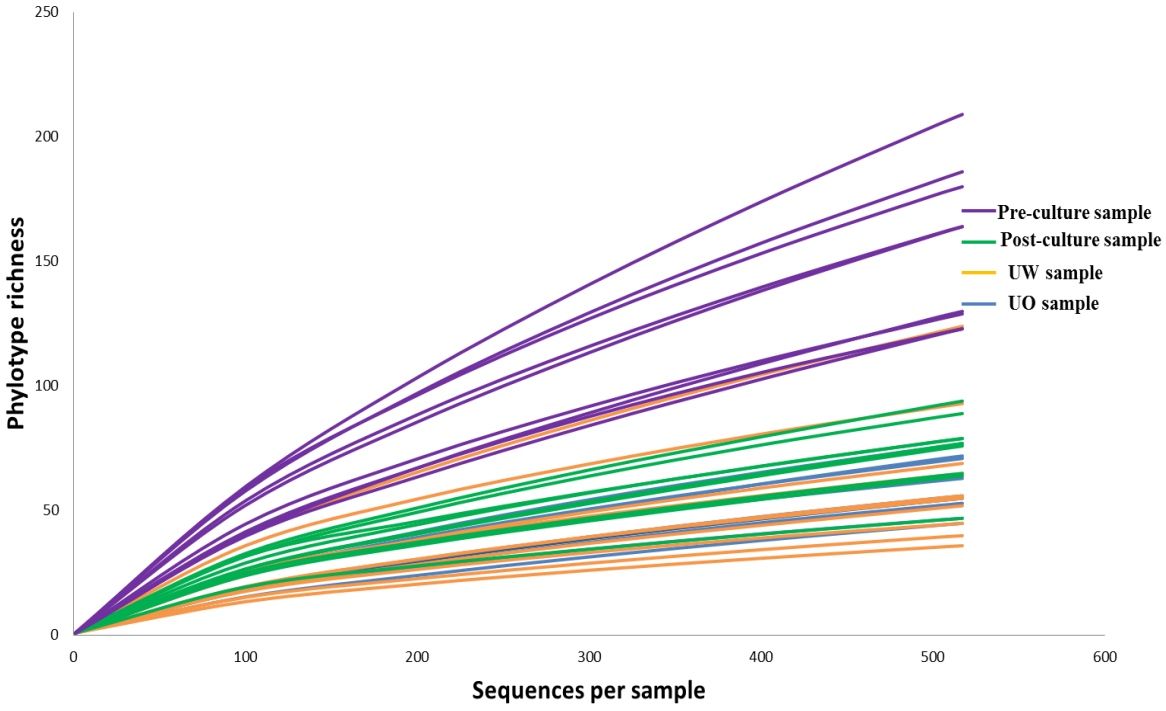
12TW	517	90	328	0.12	2.87	761
13TO	517	44	127	0.35	1.61	356
13TW	517	42	129	0.36	1.55	118
14TO	517	43	121	0.35	1.57	395
14TW	517	51	170	0.26	1.97	328
15TO	517	50	149	0.30	1.85	314
16TO	517	61	122	0.26	2.11	263
16TW	517	98	278	0.37	2.19	782
17TO	517	56	123	0.20	2.17	312
17TW	517	72	166	0.15	2.60	175
18TO	517	64	208	0.14	2.60	275
18TW	517	75	224	0.18	2.42	243
9TO	517	67	157	0.20	2.33	482
9TW	517	113	299	0.07	3.49	354
H10C	517	65	158	0.21	2.49	285
H10	517	180	344	0.02	4.45	615
H11C	517	76	204	0.10	2.87	555
H11	517	130	343	0.10	3.38	661
H12C	517	94	297	0.09	3.15	414
H12	517	164	420	0.04	4.13	763
H13C	517	79	247	0.07	3.26	539
H13	517	164	366	0.06	4.06	559
H14C	517	64	220	0.16	2.52	273
H14	517	186	405	0.03	4.34	664
H15C	517	89	181	0.09	3.11	365
H15	517	209	544	0.04	4.39	1030
H16	517	123	225	0.11	3.40	426
H17C	517	79	148	0.09	3.00	265
H18C	517	47	106	0.25	2.13	202
H18	517	123	367	0.06	3.59	845
H9C	517	77	179	0.09	2.96	336
H9	517	129	300	0.09	3.62	482
H1Cl	517	81	191	0.08	3.18	276
H1l	517	173	372	0.03	4.27	540
H2Cl	517	98	391	0.07	3.24	716
H2l	517	176	411	0.04	4.31	618
H3Cl	517	92	179	0.09	3.10	321
H3l	517	151	326	0.04	4.08	601
H4Cl	517	85	170	0.08	3.12	311
H4l	517	238	633	0.02	4.85	997
H5Cl	517	84	202	0.07	3.24	174
H5l	517	141	273	0.02	4.27	375
H6Cl	517	62	132	0.19	2.44	200
H6l	517	155	305	0.04	4.11	496
H7Cl	517	82	168	0.05	3.42	268
H7l	517	158	348	0.04	4.15	606
H8Cl	517	81	194	0.05	3.40	372
H8l	517	145	256	0.04	4.11	361


Phylotype richness
Sequences per sample
Pre-culture sample
Post-culture sample
0
50
100
150
200
250
0
100
200
300
400
500
600

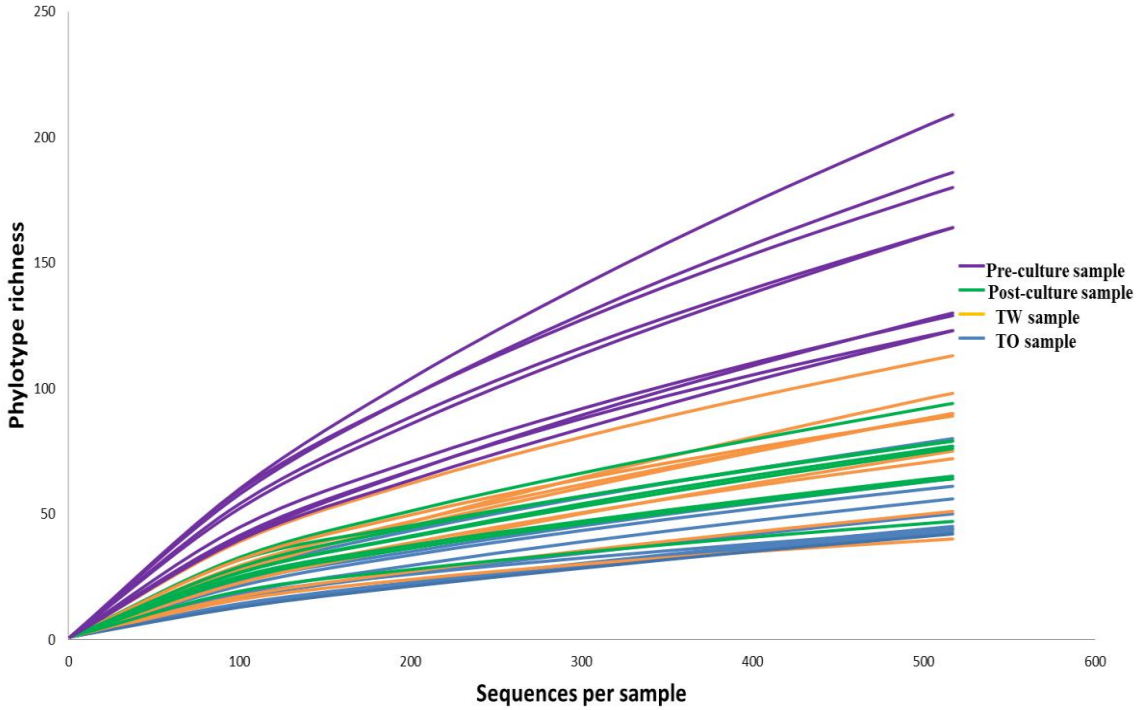
(A)


Phylotype richness
Sequences per sample
Pre-culture sample
Post-culture sample
BW sample
BO sample
0
50
100
150
200
250
0
100
200
300
400
500
600

(B)


Phylotype richness
Sequences per sample
Pre-culture sample
Post-culture sample
UW sample
UO sample
0
50
100
150
200
250
0
100
200
300
400
500
600

(C)

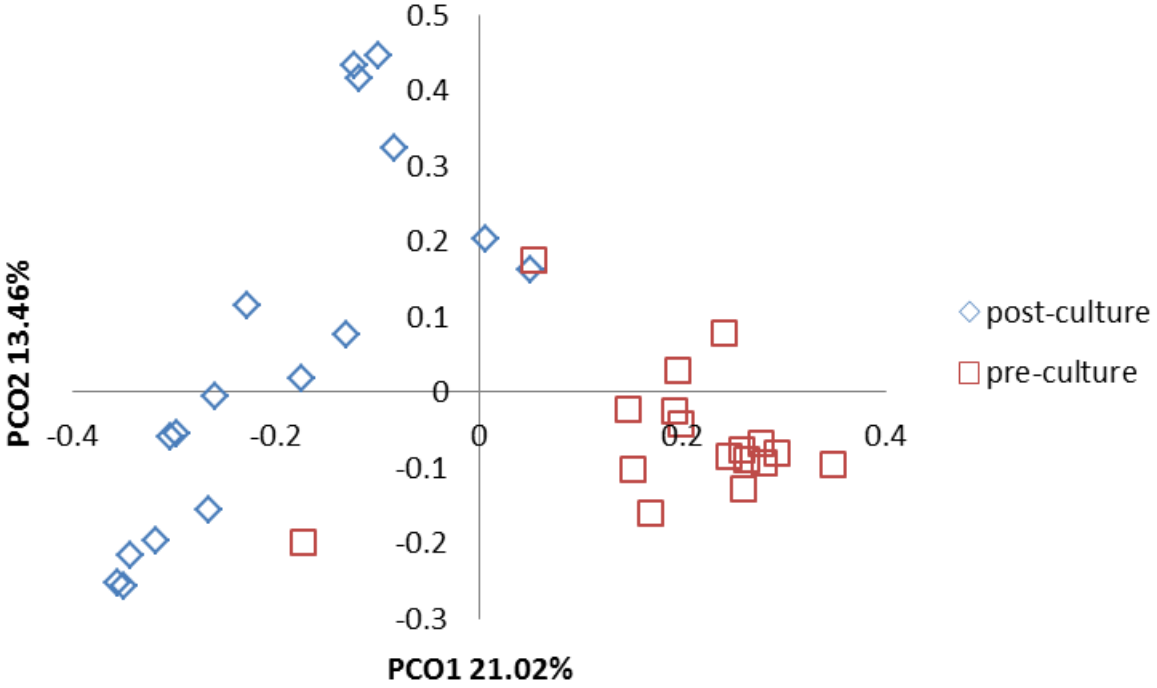

Phylotype richness
Sequences per sample
Pre-culture sample
Post-culture sample
TW sample
TO sample
0
50
100
150
200
250
0
100
200
300
400
500
600

(D)

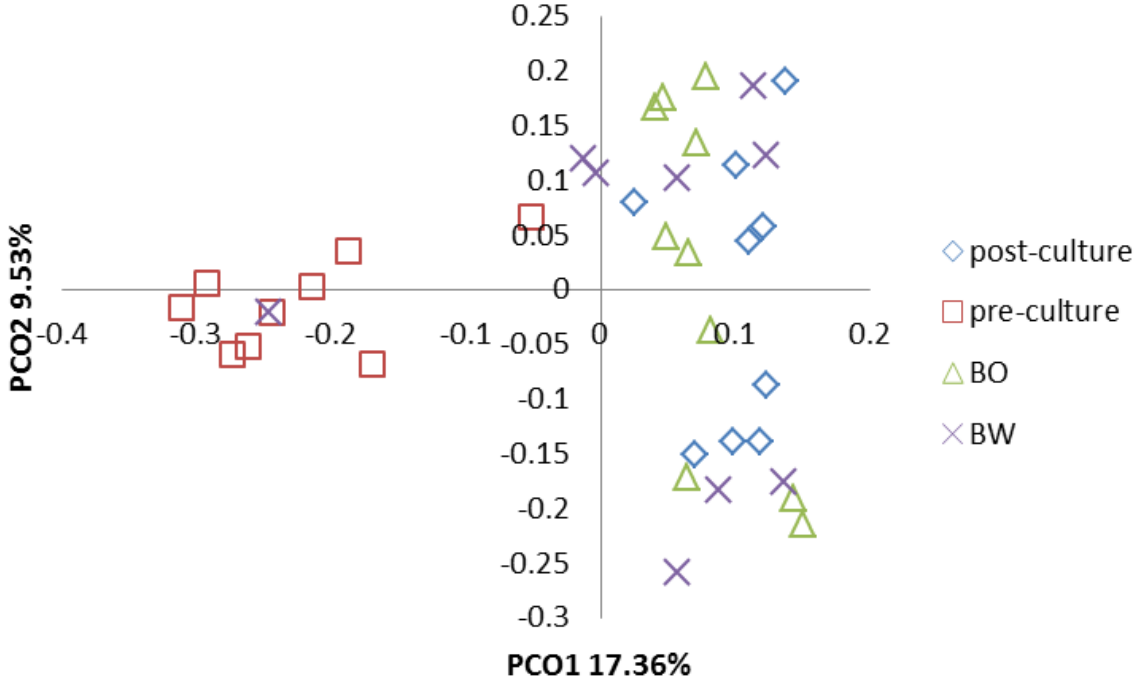
Figure 6. Rarefaction curves of different groups. (A) Rarefaction curve of pre-culture and post-culture samples; (B) Rarefaction curve of pre-culture, post-culture, BO and BW samples; (C) Rarefaction curve of pre-culture, post-culture, UO and UW samples; (D) rarefaction curve of pre-culture, post-culture, TO and TW samples. BW: samples with BAC exposure and cultured with BAC; BO: samples with BAC exposure and cultured without BAC; UW: samples with $CuSO_4$ exposure and cultured with $CuSO_4$; UO: samples with $CuSO_4$ exposure and cultured without $CuSO_4$; TW: samples with triclosan exposure and cultured with triclosan; TO: samples with triclosan exposure and cultured without triclosan; pre-culture samples: original samples without biocide exposure; post-culture samples: samples without biocide exposure and cultured without biocide.

3.4. Bacterial diversity across individual samples

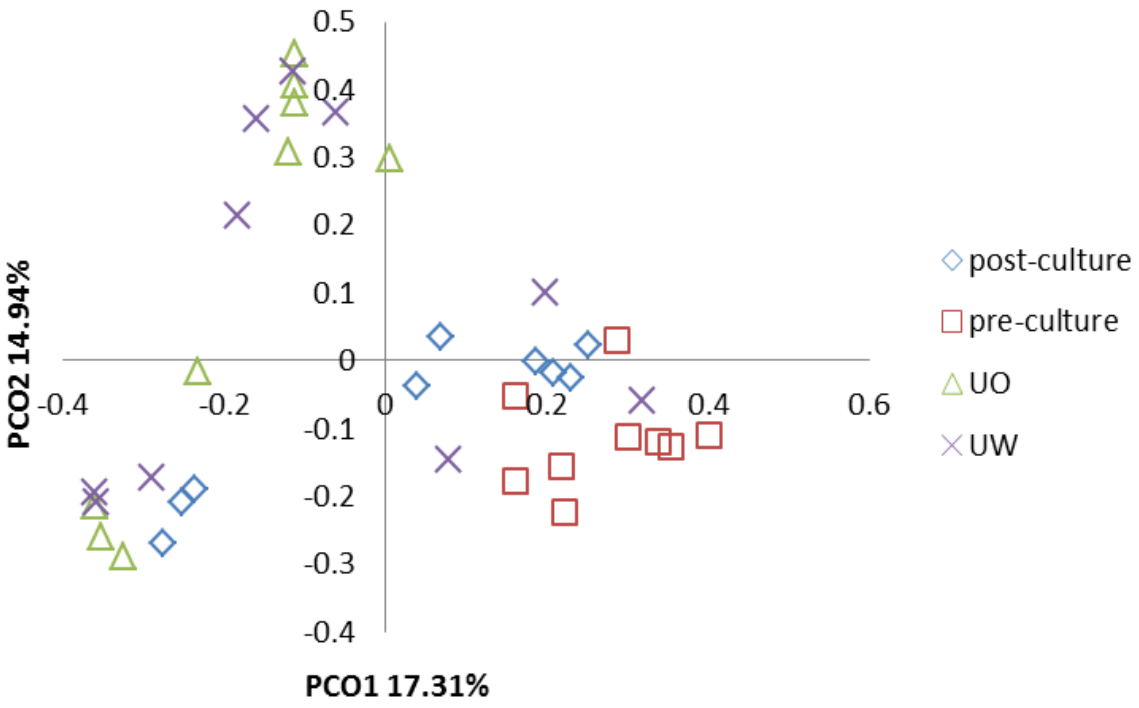
In order to view relationships among different samples based on their differences in phylogenic diversity, the beta-diversity metrics-weighted UniFrac-based principal coordinate analysis (PCoA) (Lozupone and Knight, 2005), aiming to assess the similarity among communities' structure, was used (Figure 7). There was a clear clustering by grouping of pre-culture and post-culture samples based on weighted UniFrac distance. And the Parsimony test (P-test) (Martin, 2002) was performed to confirm the significant differences in bacterial communities' structure between pre-culture and post-culture samples (p <0.001). Cluster separation was less distinct among cultured samples (post-culture samples, XO and XW samples). However, the pairwise comparison P-test also confirmed significant differences in bacterial communities' structures for post-culture samples, XO samples and XW samples (p <0.001). Most of the variations were explained by PCO1. The greatest amount of separation was observed at PCO1 (21.02%) for pre-culture and post-culture samples.


0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.2
0
0.2
0.4
PCO2 13.46%
PCO1 21.02%
post-culture
pre-culture

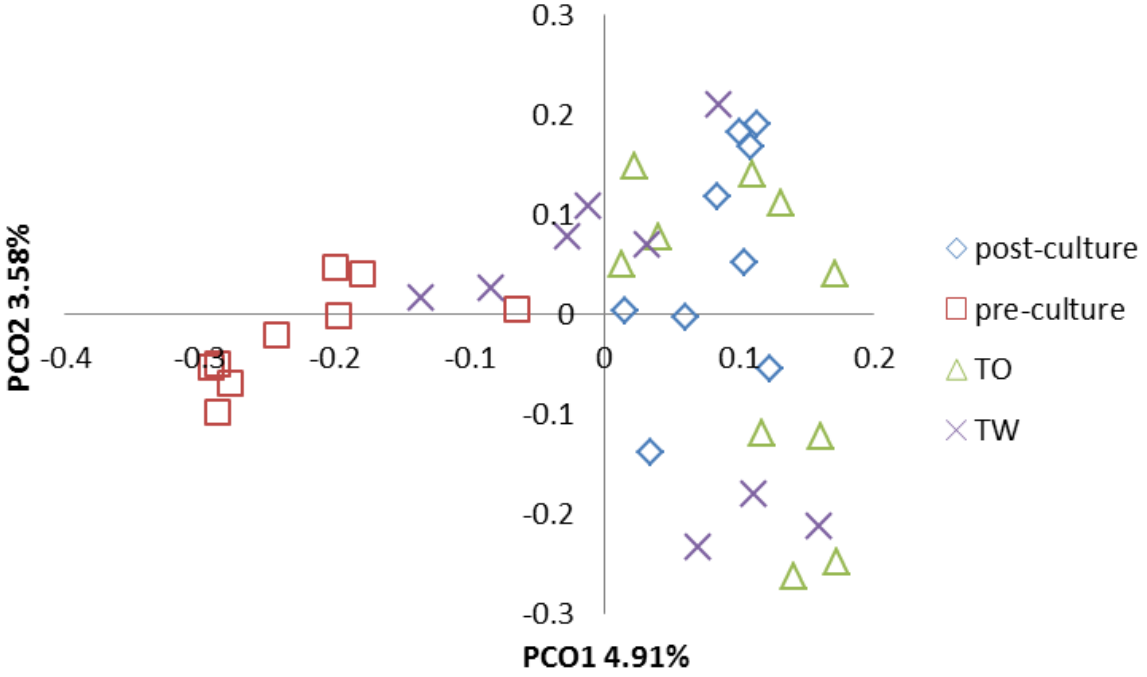
(A)


0.25
0.2
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
-0.25
-0.3
-0.4
-0.3
-0.2
-0.1
0
0.1
0.2
PCO2 9.53%
PCO1 17.36%
post-culture
pre-culture
BO
BW

(B)


0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.4
-0.2
0
0.2
0.4
0.6
PCO2 14.94%
PCO1 17.31%
post-culture
pre-culture
UO
UW

(C)



(D)

Figure 7. PCoA plots of different groups. (A) PCoA plot of pre-culture and post-culture group; (B) PCoA plot of pre-culture, post-culture, BO and BW samples; (C) PCoA plot of pre-culture, post-culture, UO and UW samples; (D) PCoA plot of pre-culture, post-culture, TO and TW samples. BW: samples with BAC exposure and cultured with BAC; BO: samples with BAC exposure and cultured without BAC; UW: samples with $CuSO_4$ exposure and cultured with $CuSO_4$; UO: samples with $CuSO_4$ exposure and cultured without $CuSO_4$; TW: samples with triclosan exposure and cultured with triclosan; TO: samples with triclosan exposure and cultured without triclosan; pre-culture samples: samples without biocide exposure and cultivation; post-culture samples: samples without biocide exposure and cultured without biocide.

3.5. qPCR results

18 pre-culture house dust samples, 18 post-culture house dust samples, and 60 biocides exposed cultured house dust samples, total 96 samples were analyzed by qPCR. There was an obvious increase of absolute DNA concentration after culturing for all 18 samples (Figure 8). Samples exposed to three different biocides were also evaluated, respectively. DNA concentrations of XO samples had the reduction ratio of 91.3±8.7% for triclosan exposure, 94.5±4.6% for BAC exposure, 97.4±2.5% for $CuSO_4$ exposure compared with post-culture samples. And for XW samples, the reduction ratios were very close to 1 among all samples. And then by combination of qPCR absolute total quantity data with relative quantity data of NGS, absolute quantity of each phylum and reduction ratio were obtained (Figure 9). Biocide BAC had better effect to *Proteobacteria* compared to the *Actinobacteria* and *Firmicutes* phyla with average reduction ratios of 96%, 92% and 72% for BW samples. *Actinobacteria* reduction ratio was the biggest among three phyla with triclosan exposure. Although for $CuSO_4$, the difference among reduction ratios for three phyla was not apparent enough, a slightly smaller reduction ratio of *Firmicutes* was observed.

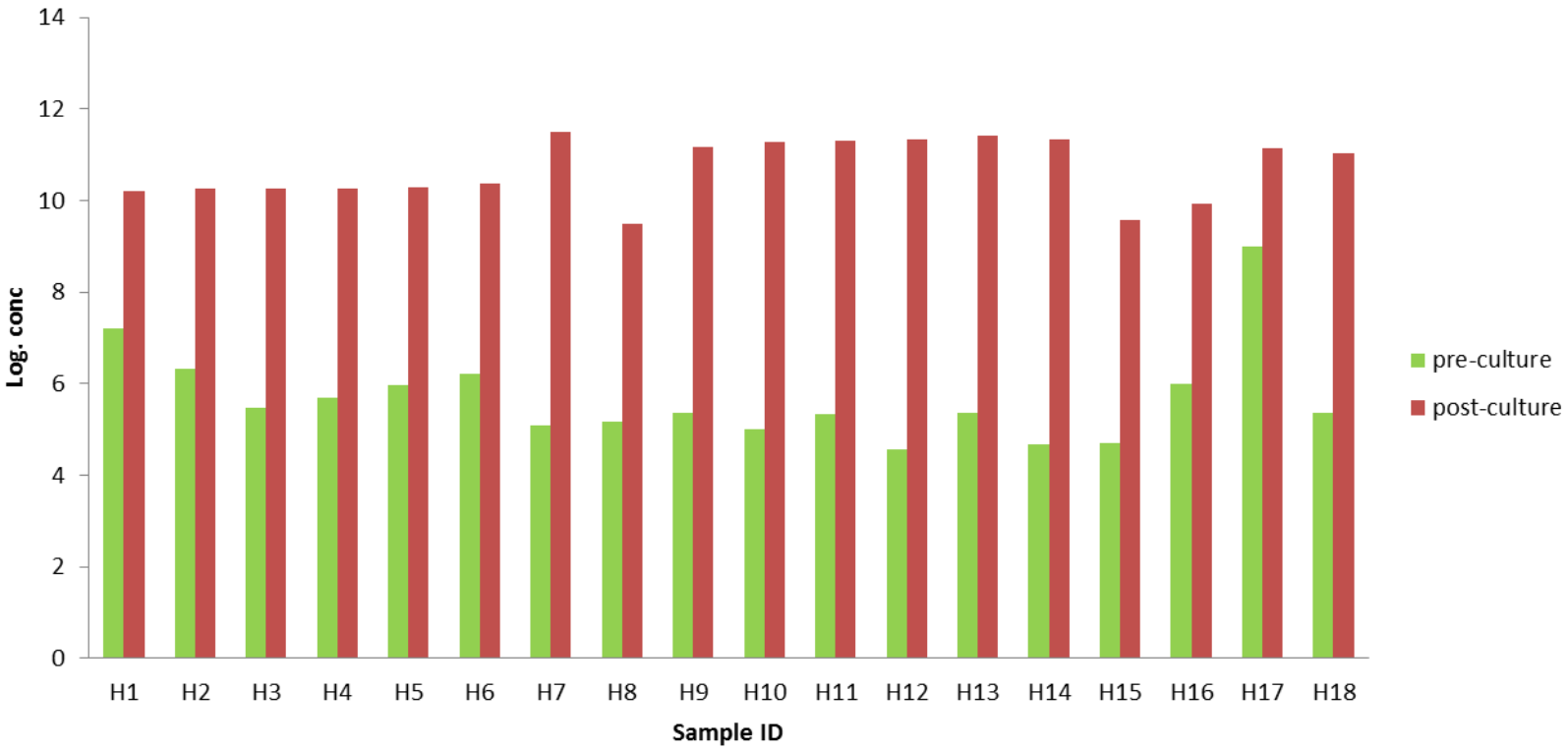
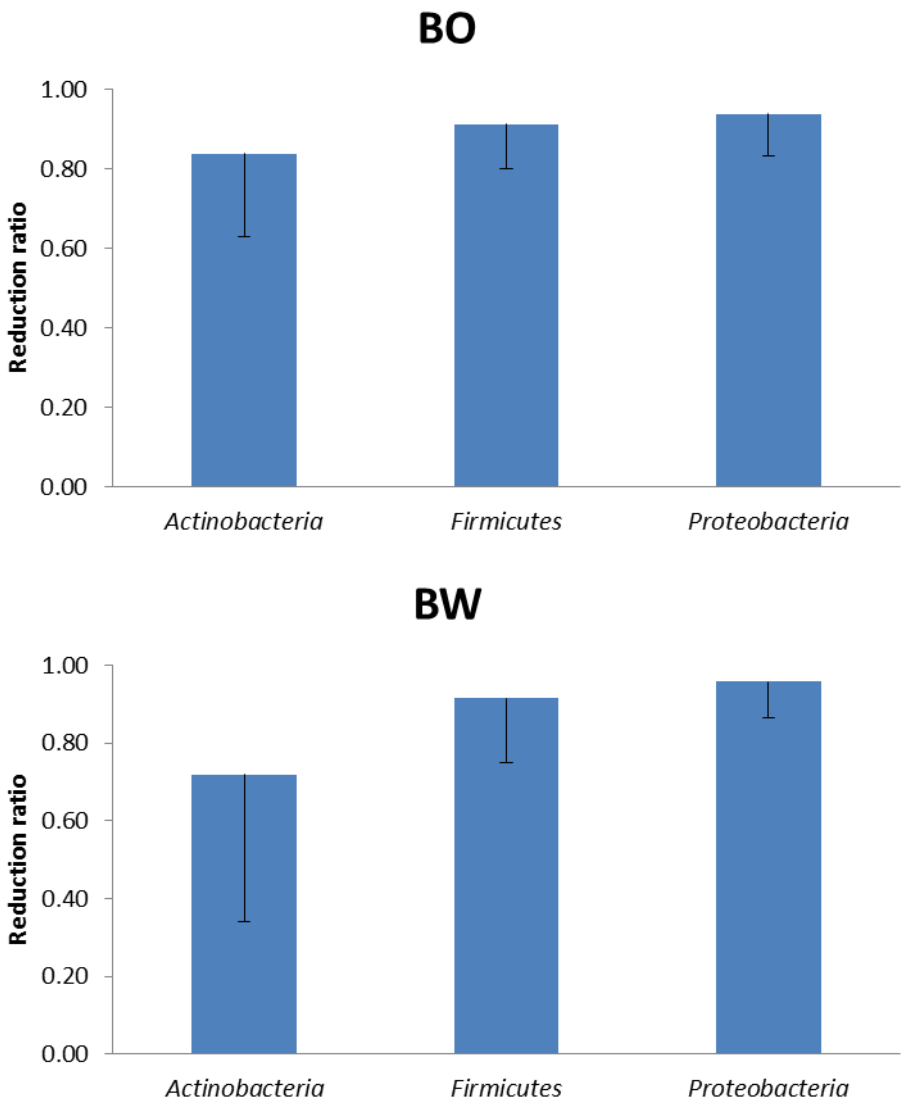
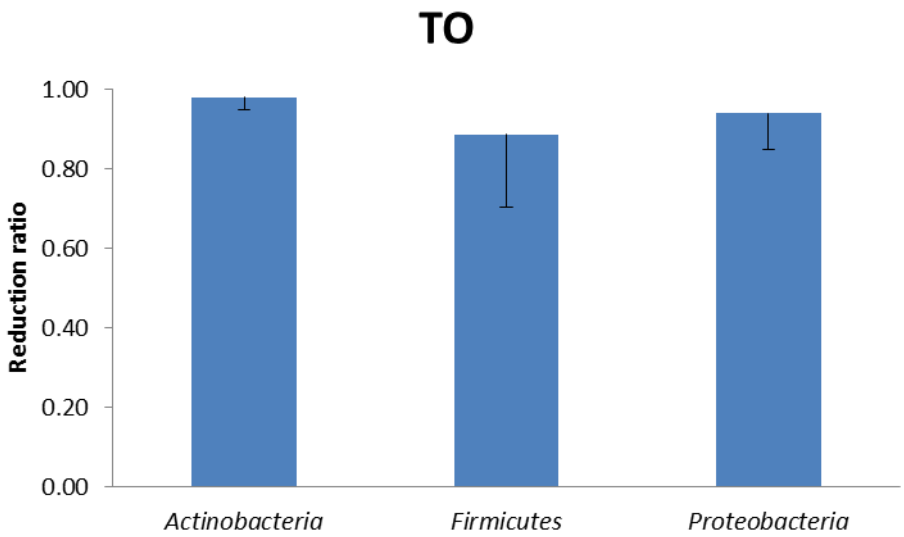
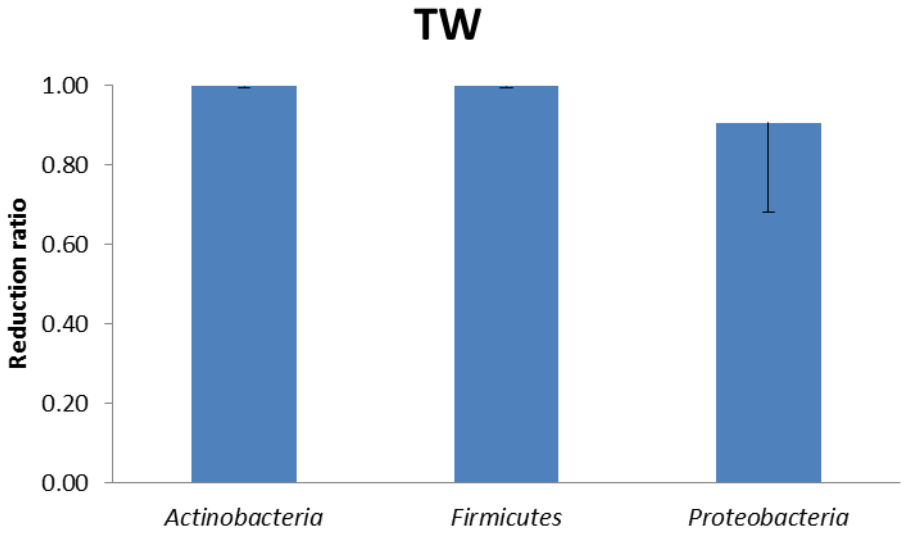


Figure 8. Comparison of bacterial concentration between pre-culture and post-culture samples. Pre-culture samples: samples without biocide exposure and cultivation; post-culture samples: samples without biocide exposure and cultured without biocide.

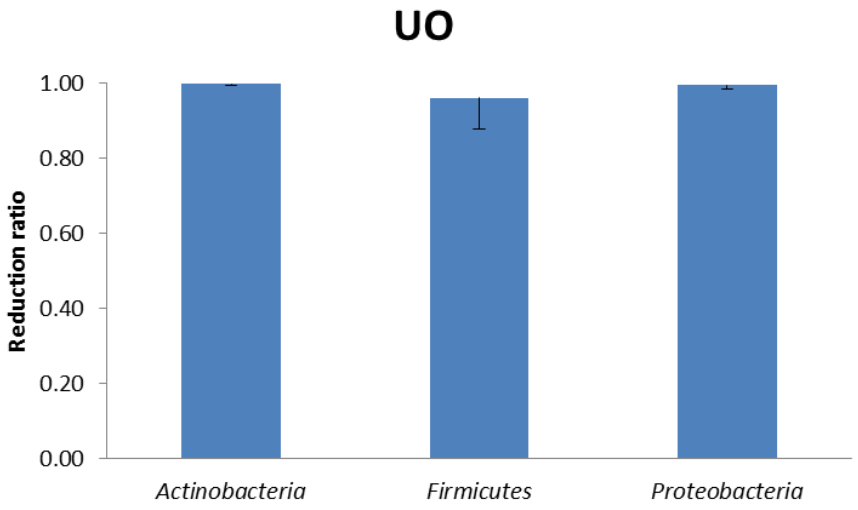

BO
1.00
0.80
0.60
0.40
0.20
0.00
Reduction ratio
Actinobacteria
Firmicutes
Proteobacteria
BW
1.00
0.80
0.60
0.40
0.20
0.00
Reduction ratio
Actinobacteria
Firmicutes
Proteobacteria

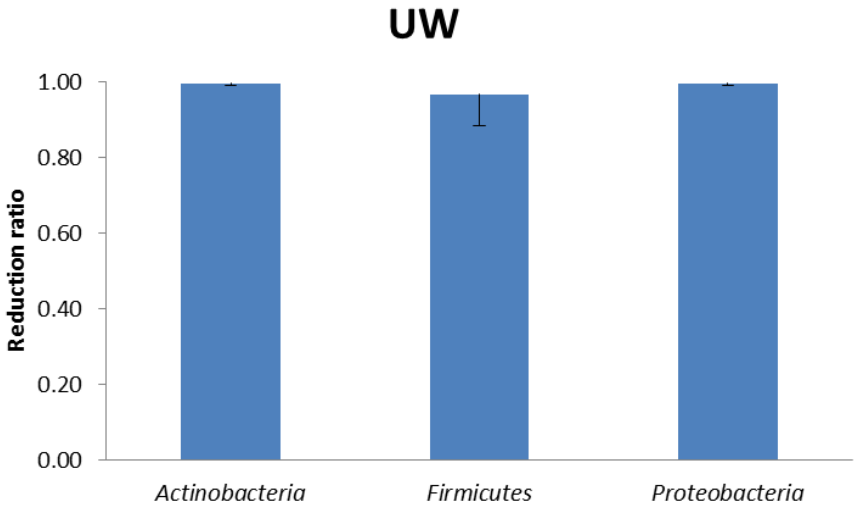
(A)


TO
Reduction ratio
1.00
0.80
0.60
0.40
0.20
0.00
Actinobacteria
Firmicutes
Proteobacteria


TW
Reduction ratio
1.00
0.80
0.60
0.40
0.20
0.00
Actinobacteria
Firmicutes
Proteobacteria

(B)





(C)

Figure 9. Reduction ratios of three main bacterial phyla after exposing to three biocides. (A) Reduction ratios of three main bacterial phyla of BO and BW samples; (B) Reduction ratios of three main bacterial phyla of TO and TW samples; (C) Reduction ratios of three main bacterial phyla of UO and UW samples. BW: samples with BAC exposure and cultured with BAC; BO: samples with BAC exposure and cultured without BAC; UW: samples with $CuSO_4$ exposure and cultured with $CuSO_4$; UO: samples with $CuSO_4$ exposure and cultured without $CuSO_4$; TW: samples with triclosan exposure and cultured with triclosan; TO: samples with triclosan exposure and cultured without triclosan; pre-culture samples: samples without biocide exposure and cultivation; post-culture samples: samples without biocide exposure and cultured without biocide.

4. Discussions

The results obtained by this study indicated that the three biocides: BAC, triclosan and $CuSO_4$, widely used in our daily life, were effective against culturable house dust-borne bacteria by applying their recommended concentrations (Figure 1). The biocide concentration could remarkably influence the biocidal activities, which was also confirmed by other studies (Grobe et. al. , 2002, Russell and McDonnell, 2000, Maillard, 2005). In addition, the culture methods (with or without biocide) also had an influence on the sanitizing results, which implying that the lasting time of biocides on targets after utilization should be noticed and studied, to reach the better sanitization effect. In a more practical way, the biocide application frequency might be an important factor to the sterilization effect. In addition, it's meaningful to notice that different biocides tended to have different biocidal effects to different bacterial phyla (Figure 9), indicating that biocide effects may be taxon-dependent. For instance, BAC had a better antibacterial behavior against *Proteobacteria* than the other two *Actinobacteria* and *Firmicutes* phyla. Triclosan was most effective against *Actinobacteria* compared to *Firmicutes* and *Proteobacteria*. While for $CuSO_4$, a slight difference could be observed that the reduction ratio was smallest of *Firmicutes*, although all the reduction ratios were close to 100%. This might offer a helpful hint about how to decide a biocide's application area depending on its targeting bacterial species, which could significantly improve the antibacterial effects. Based on these results, we can know that bacterial counts were associated with the type and concentrations of biocides applied.

The three bacterial groups based on bacterial vital status, including viable, dormant and dead, after exposure to the different concentrations of biocides were also characterized. Generally, to examine the efficacy of biocide, pure cultured bacterial strains were used to characterize the minimal inhibitory concentrations (MICs) (Vázquez-Sánchez et. al. , 2014, Meireles et. al. , 2015, Cheeseman et. al. , 2011). But we are more interested to know the bacterial vital status composition after exposure to biocides. It is scientifically significant since the dormant bacteria are still alive and can become culturable, having metabolic activities under suitable condition and bring risks to human health (Lehtinen, 2007). Certainly, the viable and dead bacteria are also important factor indicating the

biocide efficacy (Smith and Hunter, 2008). According to our results (Figure 3), dormant bacteria group mostly existed in samples exposure to lower biocide concentrations. It's noteworthy that samples having the highest concentration (0.25% v/v) of $CuSO_4$ exposure still had 12.0% dormant bacteria. This indicated that if the growth condition becomes suitable, 12.0% of the total bacteria will start to grow and have metabolic activities, potentially dangerous for human health. Thus, this may raise another important factor which we need to consider when the biocides efficacy was examined.

This study also compared the bacterial communities' composition and diversities between pre-culture and post-culture samples using Illumina NGS technology. The *Proteobacteria*, *Firmicutes*, and *Actinobacteria* were found to be the dominant phyla in house dust samples (Figure 4), which is consistent with some other studies (Rintala et. al. , 2012, Taubel et. al., 2009). However, in the post-culture samples, the abundance (relative to all phylum) of *Actinobacteria* substantially decreased among all samples, which possibly due to the fact that the most prevalent lineages acl and aclV have remained difficult to culture to date (Ghai et. al. , 2012). The diversity of pre-culture samples was considerable, but the lack of diversity at the phylum-level of post-culture samples indicated that the cultivation strongly regulated the abundance of many phyla. Measuring of the alpha diversity also indicated the richness of bacterial diversity of pre-culture samples was more than that of post-culture samples (Figure 6). Beta-diversity, particularly phylogeny-based UniFrac weighted distance, represented a means to test the similarity across different samples. The degree of similarity between bacterial communities was largely related to the cultivation ($p < 0.001$), suggesting that the same house dust samples had clearly different bacterial communities structures after cultivation.

The bacterial communities' composition and diversities were characterized using NGS technology to study the variations due to the exposure to three different kinds of biocides. In the samples with biocides exposure, *Proteobacteria* and *Firmicutes* were the dominant phyla (Figure 5). The diversity of post-culture samples was much larger compared to the samples having triclosan and $CuSO_4$ exposure, which was also proved by alpha-diversity test (Figure 6). However, alpha diversity of samples with BAC exposure was a little higher than that of post-culture samples, though all these samples alpha diversity was much lower than pre-culture samples. One of the possible reasons here is that the BAC concentration used in the NGS experiment was too low to kill or inhibit most of the

bacteria. When the Parsimony test (Martin, 2002) was applied to compare the structures of the bacterial communities from different samples, statistically significant differences were identified ($p < 0.001$), indicating that the bacterial communities became very different after exposure to the biocides. The “biodiversity hypothesis” holds that reduced contact of people with natural environmental features and biodiversity may adversely affect the human commensal microbiota and its immunomodulatory capacity, increasing prevalence of allergies and other chronic inflammatory diseases (Hanski et. al. , 2012). Decrease in microbial diversity means that beneficial bacterial are possibility being eliminated. From this biodiversity view, decreased bacterial diversities after exposure to biocides should be considered when performing the biocides utilization in indoors. Additionally, the majority of studies conducted to investigate the biocide efficacies, whereas decreased bacterial diversity caused by biocide application should not be ignored at the same time. Thus, this study raises the question about the decrease of bacterial diversity in response to the biocides application indoors, which may bring potential risks for human health, such as allergic diseases and asthma.

5. Conclusion

This study provided the comprehensive characterization of the impacts of biocides utilization on house dust-borne bacterial communities. As far as I know, our research is the first study to detail the impacts of various biocides on changes of house dust-borne bacterial community composition and diversity. By using NGS technology, it enabled us to specifically focus on the bacterial composition and diversity upon different disturbance factors, such as cultivation and biocide exposure. Application of biocides significantly simplified the bacterial composition, leading to a very low diversity, raising the public concern about decreased bacterial diversities due to biocide application in indoors, which potentially associated with health problems such as allergic diseases and asthma. In addition, by conventional growth-based method, the effects of three biocides used were evaluated. Three biocides basically effectively reduced the bacterial concentrations in indoors. However, the effects were greatly influenced by biocide concentration. Moreover, the selectivity of biocides was apparent among different bacterial phyla. The robustness of the bacterial community changes obtained in our study should be further strengthened by supplementing more diverse indoor environments, such as schools, office buildings and hospitals. Although there is limitation in this study, it will provide practical suggestions on effective biocide utilization and application.

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국문초록

Characterization of changes in cultured house dust-borne bacterial communities and diversities by biocides application

살균제 노출에 의한 집 먼지 배양 세균 군집의 구성과 다양성 변화의 특성

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실내 환경 대부분에 존재하는 세균(細菌) 중 일부는 건강 위해 요인이 될 수 있으며, 이들의 성장을 억제하기 위해 우리는 '살균제(殺菌劑)'를 사용하게 된다. 그러나 살균제(殺菌劑)의 무분별한 사용 또한, 직접적인 건강 위해뿐만 아니라 잠재적 건강 위해 실내 세균(細菌) 군집의 구성과 그 다양성의 변화, 약제 내성 세균(細菌) 출현 등을 야기할 수있다. 본 연구는 소비재에 광범위하게 사용되고 있는 살균제(殺菌劑) 세 종, triclosan, copper (II) sulfate (CuSO4), benzalkonium chloride (BAC)의 사용과 집 먼지 세균(細菌) 군집의 구성 및 다양성 변화의 관계를 보고자 하였으며, 이에 종래의

배양법을 기반으로 살균제(殺菌劑)와 배양된 세균(細菌)을 정량하고 차세대염기서열분석법 (NGS)으로 생산된 시료의 16S rDNA 서열들의 군집 식별, 종 분류단위(OTU) 분석을 통한 세균(細菌) 군집의 다양성 변화를 관찰하였다.

본 연구는, 유럽연합 집행위원회 (European Commission) 및 미국식품의약국 (U.S FDA)의 권장 소비재내 각 살균제 농도에 실제 배양된 세균(細菌) 번식이 완전히 억제되었으며, 이들의 1/10000 희석 농도에도 배양된 집 먼지 세균(細菌) 의 번식이 상당히 억제되었음을 확인하였다 (BAC: 37% 감소, triclosan: 50% 감소, CuSO4: 63% 감소). 살균 효능은 세균(細菌) 분류군에 따라서도 차이를 보였으며, BAC 는 Proteobacteria 군집을 억제하는 데에 (1/10000 희석 농도에서 96% 억제), triclosan 은 Actinobacteria 군집을 억제하는 데에 (1/100 희석 농도에서 100% 억제) 보다 효과적이었다. 반면, CuSO4 은 1/100 희석 농도에서 Proteobacteria (100%), Actinobacteria (97%), 및 Firmicutes (100%) 군집에 유사한 효과를 보였다. 군집의 다양성 변화 관찰을 위해 97%의 염기서열 유사성에 기초한 종 분류단위 (OTUs) 총 8,011 개 분석에서, 배양에 의해 집먼지 세균(細菌) 군집의 다양성이 감소하였으며 (OTUs 52% 감소), BAC 처리시에는 오히려 군집의 다양성이 증가하였으나 (OTUs 1% 증가), 이외 나머지 두 살균제(殺菌劑) 노출에 배양 세균(細菌)의 다양성은 감소한 것으로 나타났다 (CuSO4: OTUs 24% 감소; triclosan: OTUs 26% 감소). 이러한 배양된 세균(細菌) 군집의 다양성 변화는 살균제 처리 여부에 따라 통계적으로 유의한 차이를 보이는 것으로 확인되었다 ($p < 0.001$; parsimony method).

이로써, 본 연구 결과는 배양된 집 먼지 세균(細菌)의 정량적 억제 효과 및 군집의 구성과 그 다양성의 변화가 살균제(殺菌劑)농도와 종류에 영향을 받고 있음을 제시하여, 실내 세균(細菌) 군집의 선택적이고 효과적인 통제를 위한 살균제(殺菌劑)의

사용에 대한 이해를 돕고 이들의 무분별한 사용에 의한 실내 세균(細菌) 군집의 다양성 감소와 그로 인해 야기될 잠재적 건강 위해 가능성에 대한 우려를 제기하고 있다.

핵심어: 집 먼지, 세균(細菌), 살균제(殺菌劑), 세균 군집, 다양성

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