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Contamination of yellow-feathered broiler carcasses: Microbial diversity and succession during processing

Hang Wang[a], Xiaojie Qin[a], Si Mi[a], Xia Li[a], Xiaoling Wang[b], Weijiang Yan[c], Chunhui Zhang[a,*]

[a] *Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences, China*
[b] *Xinjiang Pagelang Food Co., Ltd, Urumchi City, Xinjiang Province of China, China*
[c] *Taikun Group Co., Ltd, Changji City, Xinjiang Province of China, China*

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ABSTRACT

The processing environment of broiler processing plants is a potential major source of bacterial contamination of broiler carcasses. This study investigated the effect of processing water and processing time on the microbial diversity of yellow-feathered broiler carcasses at select stages of slaughter during one commercial processing day using a high-throughput sequencing technique targeting the V3−V4 region of the 16S RNA gene. Our results demonstrated that *Firmicutes* and *Proteobacteria* were the dominant bacterial phyla of broiler carcasses and processing water in the chiller tank, whereas the processing water in the scalder tank contained a high abundance of *Firmicutes* and *Deinococcus-Thermus*. At the genus level, *Escherichia-Shigella* and *Streptococcus* were present on broiler carcasses with high abundances after defeathering, but their abundance decreased after washing and chilling. The bacterial community structure was revealed to become more complex at later stages of processing, as indicated by the consistent increase in microbial alpha diversity metrics (Chao 1, Shannoneven and Shannon) throughout the processing stages ($p < 0.05$). Significantly separate clustering of bacterial communities between scalder tank water and carcasses was revealed by PCoA analysis, indicating the limited effect of scalding water on the bacterial communities of broiler carcasses.

1. Introduction

The yellow-feathered broiler is a local poultry breed in Asia that is well known for the unique flavor of its meat (Qi et al., 2017; Wang et al., 2017). In China, the production (head units) of live yellow-feathered broiler in 2016 was approximately 4.0 billion, which was comparable with the production of white-feathered broiler (Zheng et al., 2017). Microbial control during commercial poultry processing is a key factor that influences the quality and potential shelf-life of poultry products; thus, the microbial load on the broiler carcasses during processing is a matter of continuing concern (Hinton et al., 2004; Nieminen et al., 2012).

The detection and enumeration of associated bacteria at various stages of commercial broiler production have been the subject of several decades of research (Aburuwaida et al., 1994; Geornaras and von Holy, 2000; Gill et al., 2006; Smulders et al., 2011; Oakley et al., 2013; Handley et al., 2018). The microbiological status of processed broiler carcasses is dependent on several factors, including the level of contamination from live birds (Kotula and Pandya, 1996) and the extent of contamination and cross-contamination among carcasses, equipment, and associated water during processing (Russell et al., 1997; Gill et al., 2006; Althaus et al., 2017). Revealing how microbial communities are structured during processing is critical for developing intervention strategies to control the microbial content of final chicken products (Kim et al., 2017). However, much of the current data regarding contamination during broiler processing is centered on bacterial pathogens, such as *Campylobacter* (Gruntar et al., 2015), *Salmonella* (Mikołajczyk and Radkowski, 2002), and *Listeria monocytogenes* (Van et al., 2005). Limited information is available regarding the microbial communities present on yellow-feathered broilers during the slaughtering process.

It is frequently assumed that the bacterial load of broiler carcasses changes over time as a result of the high level of bacteria on the processing equipment contaminating the carcasses during processing (Whyte et al., 2004). Indeed, several studies have been conducted on the influence of processing time, demonstrating the underlying importance of initial microbial loads on live broiler and slaughter processing performance (Geornaras and von Holy, 2000; Zweifel et al., 2015). However, there are only a limited number of studies that have

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[*] Corresponding author. Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences, Nongda South Road, Xibeiwang Town, Haidian District, Beijing, 100193, PR China.

E-mail address: zhangchunhui@caas.cn (C. Zhang).

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investigated the changes in bacterial communities over time in a broiler processing plant (Rothrock et al., 2016). The processing water in the scalder tank and chiller tank have often been addressed as sources of cross-contamination on carcasses that potentially affect the microbial profile of the final product (James et al., 2006; Duffy et al., 2014; Munther et al., 2016). Furthermore, evidence for the changes in microbiological community composition in the scalder and chiller tank water from poultry processing lines has been demonstrated (Rothrock et al., 2013, 2016). Although it seems reasonable that bacterial contamination of broiler carcasses would be associated with the processing water in the tanks, there is currently little quantitative information available with respect to the relationship between processing water and broiler carcasses.

In the present study, bacterial contamination of chilled yellow-feathered broiler carcasses was investigated during processing using an Illumina MiSeq platform to sequence 16S rRNA gene. The following questions were addressed: (1) How do the bacterial communities of yellow-feathered broilers shift during different processing stages? (2) Do the bacteria in the water from the scalder tank and chiller tank affect the bacterial communities of broiler carcasses? (3) Are the microbiological load and bacterial communities of the broiler carcasses dependent on the time of day that the samples were obtained in the plant? This work would lead to a better understanding of the diversity and succession of bacterial communities on yellow-feathered broilers during processing.

2. Material and methods

2.1. Sample collection

The study was conducted in a small-scale commercial broiler processing plant in Urumchi city, Xinjiang Province of China during a typical processing day in November 2017. Approximately 21 000 to 23 000 broilers are processed each day. Broilers were slaughtered by manual exsanguinating by knife, immediately followed by electrical stunning (70 V, 8 s), according to Islamic rules. After bleeding for 7 min, the carcasses were scalded at 60–63 °C for 180 s, mechanically defeathered, manually eviscerated, washed, and chilled. The scalder tank used was a single stage unit with a length of 12 m. Immersion chilling was conducted in a paddle-agitated chiller tank filled with 20 m^3 of an ice and tap water mixture (average temperature was 1 ± 1 °C). The average total chlorine level of the chiller water was 80–100 ppm. Carcasses were agitated for 10 min in a chiller tank. At each end of the processing day, the scalder and chiller tanks were cleaned and disinfected. The water levels within the tanks were continuously supplied during the processing day to maintain a minimum water level.

A total of 64 broiler carcasses at 67–68 days of age (female, each weighing 1.9 kg) and 32 water samples were examined in this study. Samples of female yellow-feathered broilers were collected twice during a *ca.* 5.5 h processing shift on one production day. The first collection was performed early on the production day (within the first 0.5 h), and the second collection was carried out after 5 h of production. Microbiological analyses of broiler carcasses were conducted at four stages of slaughter as follows: after defeathering, after evisceration, after washing and after chilling. At each processing stage and each collection time, different carcasses (n = 8) were selected randomly. The processing water samples (n = 8) were collected from ~5 cm below the surface of the final scalder tank and chiller tank, respectively. The experimental design and workflow of sample collection is shown in Fig. 1.

Carcass sampling was performed using the swabbing method. A pooled sample consisting of six sterile cotton swabs corresponding to a carcass sampling area of 60 cm^2, i.e., 12 sampling sites (two sites for each cotton swab) × 5 cm^2 per sampling site (5 × 1 cm) was collected. The rubbed sites of each carcass were selected according to Gill et al. (2006) with some modifications. The collection areas of the neck, tail, breast, back, wing, thigh and drumstick were 5 cm^2, 5 cm^2, 10 cm^2, 10 cm^2, 10 cm^2, 10 cm^2 and 10 cm^2, respectively. At each sampling site, a moistened (sterile 0.9% sodium chloride solution) swab was rubbed vertically (delineated by a template). After swabbing, the cotton swabs were placed into tubes with sterile 0.9% NaCl solution. The samples were transferred in iceboxes to the laboratory and stored at 0–4 °C and analyzed within 4 h.

2.2. Total aerobic mesophilic bacteria

For carcass samples (n = 3), six cotton swabs per carcass were placed into tubes with 60 mL of sterile 0.9% NaCl solution. The contents of the cotton swabs were extracted into the diluent by agitation on a vortex-type mixer for 1 min. Then, the extraction was serially diluted (1:10) in sterile 0.9% NaCl. For water samples (n = 3), water was serially diluted directly. The aerobic mesophilic bacteria counts were determined in plate count agar by the pour plate method. Samples (1 mL) of serial dilutions were plated onto plate count agar and incubated at 36 ± 1 °C for 48 h. Each sample was plated in duplicate. Counts of each sample were expressed as log CFU/cm^2 or log CFU/mL.

2.3. DNA extraction and 16S rRNA amplicon sequencing

Bacterial DNA was extracted as described by Zhang et al. (2017) with some modifications. For carcass samples (n = 5), six cotton swabs per carcass were placed into centrifuge tubes with 30 mL of sterile 0.9% NaCl solution. The contents of the cotton swabs were extracted into the diluent by agitation on a vortex-type mixer for 1 min. For water samples (n = 5), water was used for the DNA extraction directly. Samples extracted from swabs and water samples were centrifuged at 400 × *g* for 5 min. Supernatants were collected and then centrifuged at 10 000 × *g* for 10 min. Subsequently, the centrifugal sediment was resuspended in 1 mL of sterile 0.9% NaCl solution and transferred to centrifuge tubes. DNA was extracted from the centrifugal sediment using a bacterial DNA extraction kit (DL111-01, Bomad Biological Technology Co., Ltd., Beijing, China). The final DNA concentration and purity were determined by a NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific, Wilmington, USA). DNA quality was checked by 1% agarose gel electrophoresis.

The V3−V4 region of the 16S r RNA gene was targeted for amplification using primers 338 F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806 R (5′-GGACTACHVGGGTWTCTAAT-3′) by a thermocycler PCR system (GeneAmp 9700, ABI, USA). PCRs were performed in triplicate in 20 μL reactions containing 10 ng of template DNA, 4 μL of 5 × FastPfu Buffer, 0.8 μL of each primer (5 mM), 2 μL of 2.5 mM dNTPs, and 0.4 μL of FastPfu Polymerase. PCR was carried out with an initial denaturation step at 95 °C for 3 min, followed by 27 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 45 s, with a final extension step of 72 °C for 10 min. The amplified product was visualized on 2% agarose gel and further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA). The concentration of each PCR product was quantified using QuantiFluor™ -ST (Promega, USA) according to the manufacturer's protocol.

PE amplicon libraries were constructed, and purified amplicons were pooled in equimolar and paired-end sequenced (2 × 300 bp) on an Illumina MiSeq platform (Illumina, San Diego, USA), which was conducted by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The sequence data generated in this study was submitted to the NCBI short reads archive (SRA) database as BioProject PRJNA479927.

2.4. Processing of sequencing data

Raw fastq files were demultiplexed, quality-filtered by Trimmomatic and merged using FLASH with standard criteria (Song et al., 2017). Sequences were clustered into operational taxonomic units (OTUs) defined by 97% similarity based on UPARSE (version 7.1

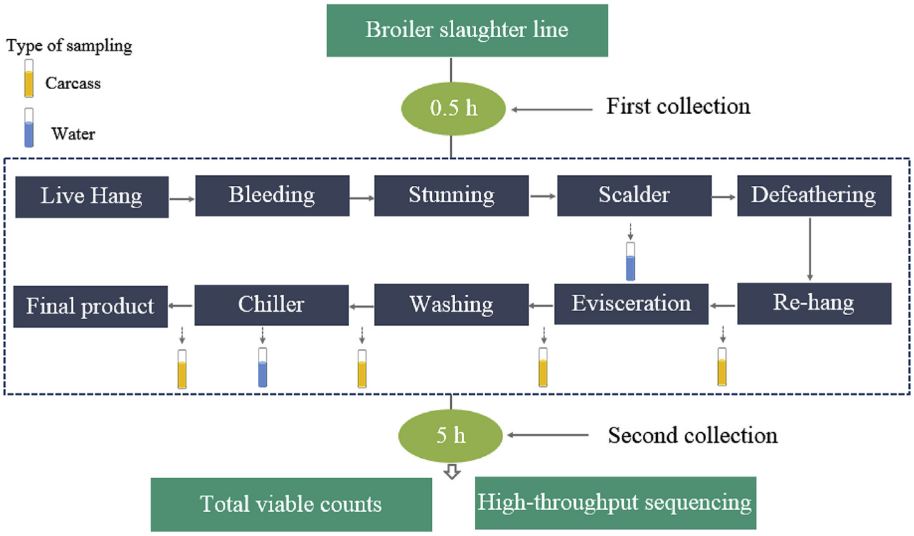

Fig. 1. Experimental design and workflow of sample collection.

http://drive5.com/uparse/). Chimeric sequences were identified and removed by UCHIME. The taxonomy of each 16S rRNA gene sequence was analyzed by the Ribosomal Database Project (RDP) Classifier algorithm (http://rdp.cme.msu.edu/) against the Silva (SSU123) 16S rRNA database using a confidence threshold of 70%. Alpha diversity was calculated by Mothur (Version 1.30.1) to describe the microbial richness, diversity and coverage within samples using the Chao1 index, Shannon–Wiener diversity index and Good's coverage index, respectively.

2.5. Statistical analysis

All measurements were expressed as the mean ± standard deviation. Differences in total aerobic mesophilic bacteria and alpha diversity characteristics among different processing stages were explored by one-way Duncan's ANOVA procedure using SPSS 19.0 at $p < 0.05$. Differences between early and end sampling groups were explored by *t*-test with significance at $p < 0.05$. Venn diagrams were performed using the R package (version 3.3.1) to show unique and shared genera among broiler carcasses and water samples. Principal coordinate analyses (PCoA) was conducted to compare similarities among carcasses and water samples. In Venn and PCoA models, each sample group was including replicate individuals from both early and late sampling time (n = 10).

3. Results

3.1. Total aerobic mesophilic bacteria

Early in the processing day, the counts of aerobic mesophilic bacteria on carcasses was 5.2 log CFU/cm^2 after defeathering (Fig. 2A). Evisceration did not cause significant changes in the aerobic mesophilic counts early in the processing day, and the aerobic mesophilic counts on carcasses decreased from 4.9 log CFU/cm^2 to 3.8 log CFU/cm^2 ($p < 0.05$) after chilling. When the carcass samples obtained at the end of the processing day were examined, a significant increase in aerobic mesophilic bacteria was observed after evisceration. Similarly, a significant reduction in the aerobic mesophilic bacteria counts was also observed after washing and chilling ($p < 0.05$). With regard to the processing time, there were significantly higher counts of bacteria on carcasses after evisceration and chilling at the end of the production day compared to on carcasses early in the production day ($p < 0.05$) (Fig. 2B). In terms of water samples, the bacterial counts in both the scalder and chiller tanks increased significantly throughout the

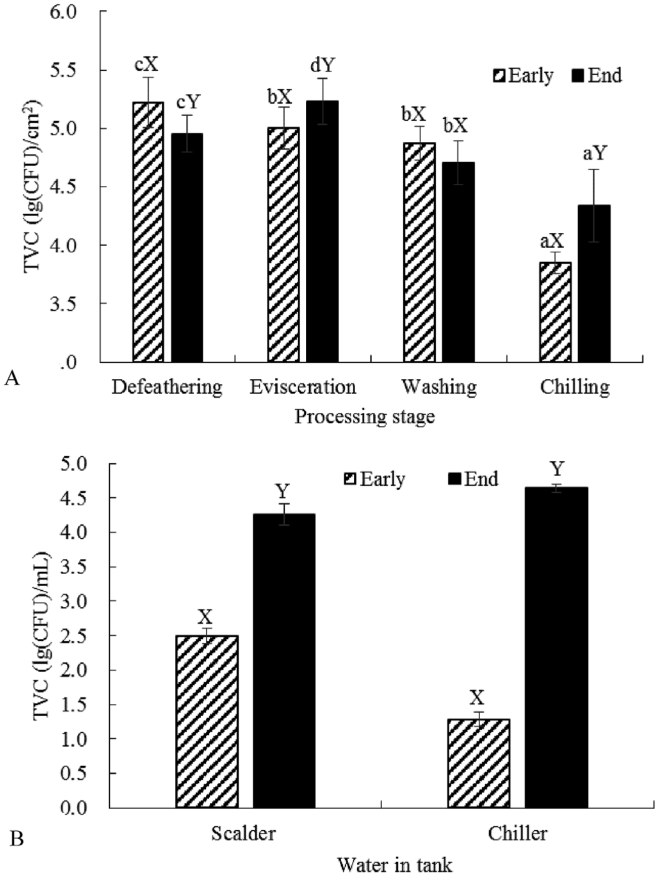

Fig. 2. Total aerobic mesophilic bacteria of the broiler carcass surfaces during general processing stages (A) and processing water in the scalder and chiller tanks (B) at different processing times. Means with standard deviation (n = 3) are shown. Different lowercase letters (a, b) indicate significant difference ($p < 0.05$) of means between different process stages; different uppercase letters (X, Y) indicate significant difference ($p < 0.05$) of means between the two sampling periods within the same process or water sample. Early, samples collected early in the processing day; End, samples collected at the end of the processing day.

Table 1
Alpha diversity characteristics of broiler carcass surfaces and processing water in scalder and chiller tanks.

Alpha Diversity indices	Sampling time	Processing stages					
		Defeathering	Eviscerating	Washing	Chilling	Water of scalder tank	Water of chilling tank
Richness (Chao1)	Early	750.6 ± 23.2^{aX}	869.0 ± 66.2^{bX}	861.6 ± 68.2^{bX}	955.8 ± 28.7^{cX}	702.5 ± 50.7^{aX}	840.6 ± 91.9^{cX}
	End	680.4 ± 61.5^{aY}	762.2 ± 28.0^{bcY}	803.3 ± 35.8^{cX}	945.4 ± 90.4^{dX}	713.3 ± 35.9^{abX}	814.8 ± 41.2^{dX}
Evenness (Shannoneven)	Early	0.54 ± 0.02^{aX}	0.63 ± 0.05^{abX}	0.66 ± 0.02^{bX}	0.65 ± 0.03^{bX}	0.59 ± 0.02^{abX}	0.62 ± 0.14^{abX}
	End	0.56 ± 0.06^{aX}	0.55 ± 0.02^{aY}	0.61 ± 0.01^{bY}	0.67 ± 0.04^{cX}	0.60 ± 0.01^{bX}	0.71 ± 0.01^{cX}
Diversity (Shannon)	Early	3.5 ± 0.1^{aX}	4.1 ± 0.4^{bX}	4.3 ± 0.1^{bX}	4.4 ± 0.2^{bX}	3.8 ± 0.1^{abX}	4.2 ± 1.0^{dX}
	End	3.5 ± 0.4^{aX}	3.5 ± 0.1^{aY}	3.9 ± 0.1^{bY}	4.5 ± 0.3^{cX}	3.8 ± 0.1^{bX}	4.8 ± 0.1^{eX}
Coverage (%)	Early	99.6 ± 0.05	99.6 ± 0.07	99.6 ± 0.09	99.6 ± 0.02	99.7 ± 0.03	99.7 ± 0.05
	End	99.6 ± 0.05	99.6 ± 0.04	99.6 ± 0.02	99.6 ± 0.05	99.7 ± 0.02	99.6 ± 0.10

Data represents as mean ± standard deviation (n = 5). Chao 1, Shannoneven, Shannon, and Good's coverage were calculated at 3% distance. Means with the same lowercase letters (a-d) within rows do not differ ($p > 0.05$); Means with the same uppercase letters (X,Y) within columns for each parameter do not differ ($p > 0.05$). Early, samples collected at early processing day; End, samples collected at end processing day.

processing day ($p < 0.05$).

3.2. Validation of the sequencing accuracy

A total of 3 101 418 high-quality reads were obtained from 60 samples (36 364 to 66 435 reads per sample). At 97% sequence identity, high-quality sequences were clustered into 1373 OTUs. Assessment of rarefaction curves based on the Shannon biodiversity index indicated that all the curves tended to reach a plateau (Fig. S1). Therefore, all the obtained sequencing data were deemed adequate to cover the vast majority of biodiversity contained within the detected samples. This consideration was further supported by the observed values of Good's coverage estimator (greater than 99.5%) for all the samples (Table 1).

3.3. Community structure and diversity

The major bacterial community indices are shown in Table 1. Generally, the richness (Chao 1), evenness (Shannoneven) and diversity index (Shannon) of bacterial communities from carcass samples increased consistently through processing stages ($p < 0.05$) and reached the highest richness and diversity after chilling. After chilling, the richness, evenness and diversity of bacterial communities on carcasses did not change throughout the processing day ($p > 0.05$). In terms of both chiller tank and scalder tank, there were no significant differences in bacterial richness, evenness and diversity of the processing water between the two processing periods ($p > 0.05$).

A Venn diagram revealed that 248 taxa, which represented 49.7% of the total genera, were common to all samples (Fig. 3 A). Water samples from the chiller tank and scalder tank contained the highest unique genera, with 45 and 10, respectively. With respect to carcasses after chilling, 401 genera were shared between the carcass and chiller water samples, whereas only 264 genera were shared between the carcass and scalder water samples (Fig. 3 B and C).

The principal coordinate analysis (PCoA) with a weighted UniFrac matrix indicated that bacterial communities on carcasses from different processing stages clustered tightly (Fig. 4). Significantly separated clustering of bacterial communities was revealed between scalder tank water and the carcasses, with main principal component (PC) scores of PC1 = 58.09% and PC2 = 10.86%.

3.4. Relative abundance of major phyla and genera

In total, 29 phyla were identified, among which a selection of the relevant top 7 (> 1% of total OTUs), *Firmicutes*, *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Deinococcus-Thermus*, *Thermotoga*, and *Tenericutes,* were represented (Fig. 5A). *Firmicutes* were the predominant phylum, representing 33.4%–48.0% of the bacterial communities in carcass samples, 53.7%–64.3% of the communities in the scalder tank water, and 55.4%–58.8% of the communities in the chiller tank water. In carcass samples, *Proteobacteria* was the second most abundant phylum, whereas *Deinococcus-Thermus* (15.3%–19.7%) and *Thermotogae* (1.1%–11.8%) were dominant and only identified in the

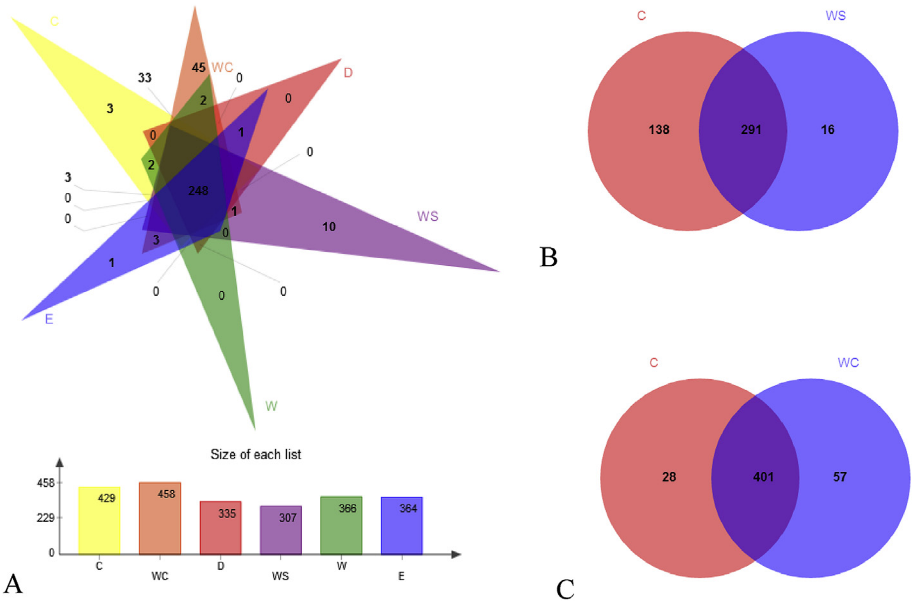

Fig. 3. Venn diagrams for numbers of shared and unique genera among broiler carcasses and processing water samples (n = 10). (A) Unique and shared genera present in all samples; (B) Unique and shared genera between scalder water and carcasses after chilling; (C) Unique and shared genera between chiller water and carcasses after chilling. D, carcasses after defeathering; E, carcasses after evisceration; W, carcasses after washing; C, carcasses after chilling; WS, processing water from the scalder tank; WC, processing water from the chiller tank.

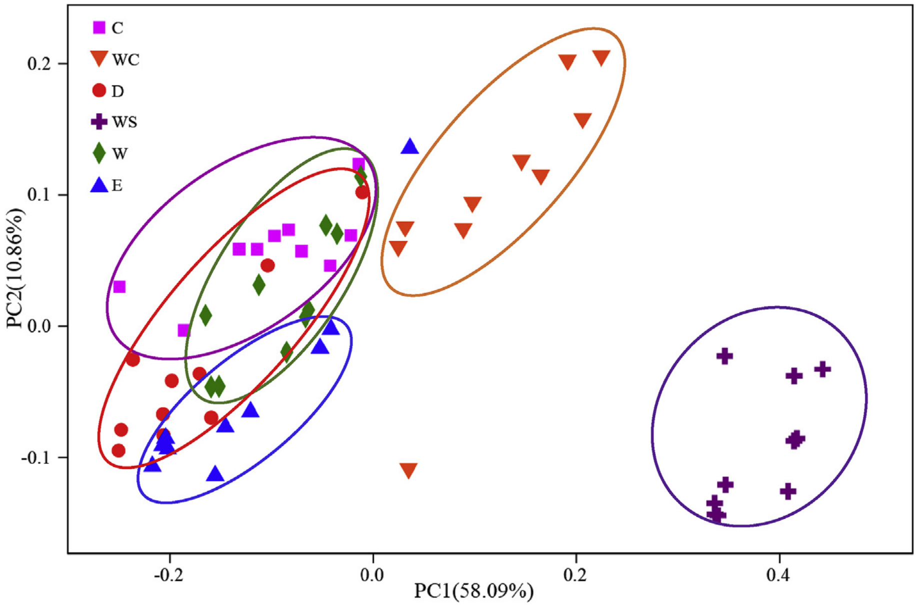

Fig. 4. Principal coordinate analysis (PCoA) of bacterial communities across carcasses surfaces and processing water samples (n = 10). E, carcasses after evisceration; W, carcasses after washing; C, carcasses after chilling; WS, processing water from the scalder tank; WC, processing water from the chiller tank; Early, samples collected early in the processing day; End, samples collected at the end of the processing day.

scalder tank water samples. Overall, microbiota on carcasses from different processing stages revealed generally similar patterns at the phylum level, except that the abundance of the *Proteobacteria* phylum decreased after washing at the end of the processing day. Compared to the carcasses obtained at the early processing time, the abundance of the *Proteobacteria* phylum was higher for carcasses obtained at both defeathering and evisceration stages late in the processing day.

The total reads corresponded to 203 families and 499 different genera. At the genus level, bacterial communities of carcasses were dominated mainly by *Lactobacillus*, *Rothia*, *Escherichia-Shigella*, *Acinetobacter*, and *Streptococcus* (Fig. 5B). A heatmap of the top 50 genus-level phylotypes was constructed and showed differences in bacterial composition and dynamics during broiler processing (Fig. 6). The relative abundance of genera is depicted by color intensity from green (lowest relative abundance) to red (the highest relative abundance). The increase in the abundance of *Lactobacillus* on carcasses after evisceration was observed both at the early and late processing stages of the day. Relative to the early processing time point in the day, the abundance of *Rothia* was decreased to 9.1% after evisceration. Along with the processing stages, the relative abundance of *Escherichia-Shigella* and *Streptococcus* decreased, reaching their lowest level after chilling. *Acinetobacter* on broiler carcasses displayed an inverse profile to *Escherichia-Shigella* and *Streptococcus*, being present at a relatively low level after defeathering and then steadily increasing throughout each processing stage. In the scalder tank, *Lactobacillus* (21.1%–12.7%), *Thermus* (14.9%–19.4%), *Blautia* (3.8%–15.6%) and *Fervidobacterium* (1.1%–11.8%) were dominant. The relative abundance of *Fervidobacterium* in the scalder tank water was higher early in the processing day, whereas it decreased from 11.8% to 1.1% at the end of the processing day. In contrast, the abundance of *Faecalibacterium*, *Thermus*, and *Blautia* increased throughout the processing day. In the chiller tank water, the abundance of *Acinetobacter and Streptococcus* was higher for the samples obtained late on the processing day, while *Bacteroides* decreased.

4. Discussion

In the present study, we demonstrated that the bacterial communities of yellow-feathered broiler carcasses mainly consisted of *Firmicutes*, *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes* phyla, confirming previous reports that were related to poultry (Kim et al., 2017; Rothrock et al., 2016). The bacterial community structure was revealed to become more complex at later stages of processing, as indicated by the consistent increase of microbial alpha diversity metrics (Chao 1, Shannoneven and Shannon) throughout the processing stages ($p < 0.05$). The results of the bacterial richness analysis (Chao 1, Table 1) on carcasses led to the identification of evisceration and chilling as the two main carcass contamination points. Evisceration can lead to further contamination of carcasses, particularly with fecal bacteria if the intestinal tract is ruptured during the process (Russell and Walker, 1997). The contamination during the evisceration process in the present study may involve two aspects, namely, contamination by new species or the increased number of bacteria on the carcasses (Fig. 2A). Increases in the relative abundances of *Lactobacillus* (9.3%–20%) and *Ruminococcus* (0.8%–4.0%) were observed after the evisceration process early in the processing day. This is in accordance with the literature, which revealed that these species are major microorganisms originating from the broiler gastrointestinal tract (Mancabelli et al., 2016; Ranjitkar et al., 2016). In fact, the operation of evisceration was carried out manually in this factory, which may have led to a high risk of carcass contamination by operator handling or improperly cleaned tools (Matias et al., 2010).

Immersion chilling is carried out to limit the growth of pathogenic and spoilage microorganisms by reducing the temperature of the poultry. Chilling may also influence the bacterial load of the immersion-chilled poultry carcasses (James et al., 2006), which depends upon the volume of water in the chiller, the ratio of carcasses to water, and the bacterial load present on the carcasses before chilling (Northcutt et al., 2006). The positive effect of water chilling on the mesophilic bacterial level (Fig. 2) supported the result of a previous study by Berrang et al. (2008), who reported a significant reduction of aerobes on carcasses after chilling. This reduction is due to the efficacy of chlorination (80–100 ppm) in the chiller tank. However, the taxonomic diversity increased after the chilling process (429 genera after chilling vs. 366 genera after washing, Fig. 3). Thus, even if the chilling process had the effect of reducing the bacterial load of the carcasses, cross-contamination still occurred when the boilers were immersed in the chiller tank. The cross contamination in immersion chiller tanks has previously been noted, which might be explained by considering the disruption of the digestive tract during evisceration (Duffy et al., 2014). Kim et al. (2017) showed that earlier processing stage samples exhibited greater OTUs compared to samples after chilling. The use of sprays that contained biocides such as Amplon (a blend of sulfuric acid and sodium sulfate) or peracetic acid for chilling may be the reason for

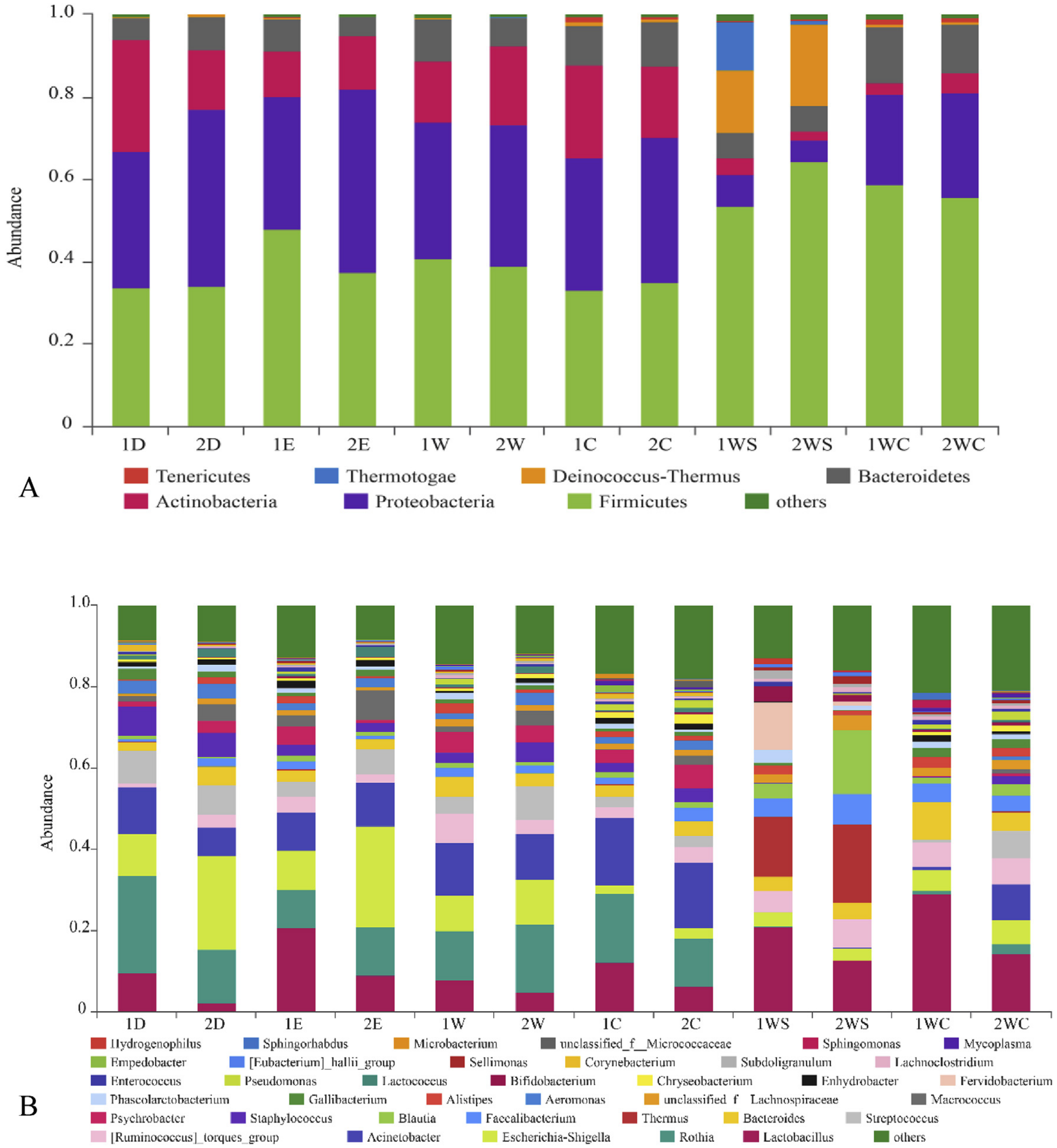

Fig. 5. Relative abundance (%) of major bacteria among different groups on broiler carcass surfaces and processing water in tanks at the phylum (A) and genus level (B) (n = 5). D, carcasses after defeathering; E, carcasses after evisceration; W, carcasses after washing; C, carcasses after chilling; WS, processing water from the scalder tank; WC, processing water from the chiller tank; the numbers 1 and 2 before letters represent samples collected at the beginning and end of the processing day, respectively. Relative abundance is expressed as the mean value.

this variation. Further investigations may need to cover the influence of the chilling system, chlorine concentration, temperature of the chilling water, and the immersion time on the microbial communities of broiler carcasses.

Acinetobacter is widely regarded as one of the dominant microbial species present on poultry and in the meat processing environment (Lupo et al., 2014; Stellato et al., 2016). A recent finding demonstrated that *Acinetobacter* was a genus with high abundance initially present in yellow-feathered broiler carcasses (Wang et al., 2017). *Acinetobacter* can grow and dominate spoilage microorganisms during the air-packaged storage of broiler meat (Zhang et al., 2012). It was hypothesized that the slaughterhouse environment is contaminated with *Acinetobacter* from broilers during processing, leading to a high abundance of *Acinetobacter* in the chiller tank at the end of the processing day. During processing, we observed a significant decrease in the *Proteobacteria* phylum, which was mainly attributed to the decrease of *Escherichia-Shigella*. Our results indicated that the washing process, as well as the immersion chilling process, could influence the attachment of *Escherichia-Shigella* to broiler carcasses due to continuous replenishment and exchange of the carcass surface water film (Lehner et al., 2014). Similarly, the abundance of the genus *Streptococcus* within the Streptococcaceae family decreased with the processing stages.

Previous studies have reported that *Pseudomonas*, *Aeromonas*, *Lactobacillus*, and *Streptococcus* were the main spoilage bacteria during the storage of poultry/poultry products (Wang et al., 2017; Zhang et al., 2012). However, *Pseudomonas* was found at low abundance on both carcass and water samples in the present study. This finding is consistent with the results reported by Wang et al. (2017), who observed that the growth of *Pseudomonas* on broiler carcasses may be absent or present at low abundance levels initially. Our results provided evidence that *Aeromonas* and *Streptococcus* were highly abundant on carcasses after defeathering, while they were present at low abundance levels in

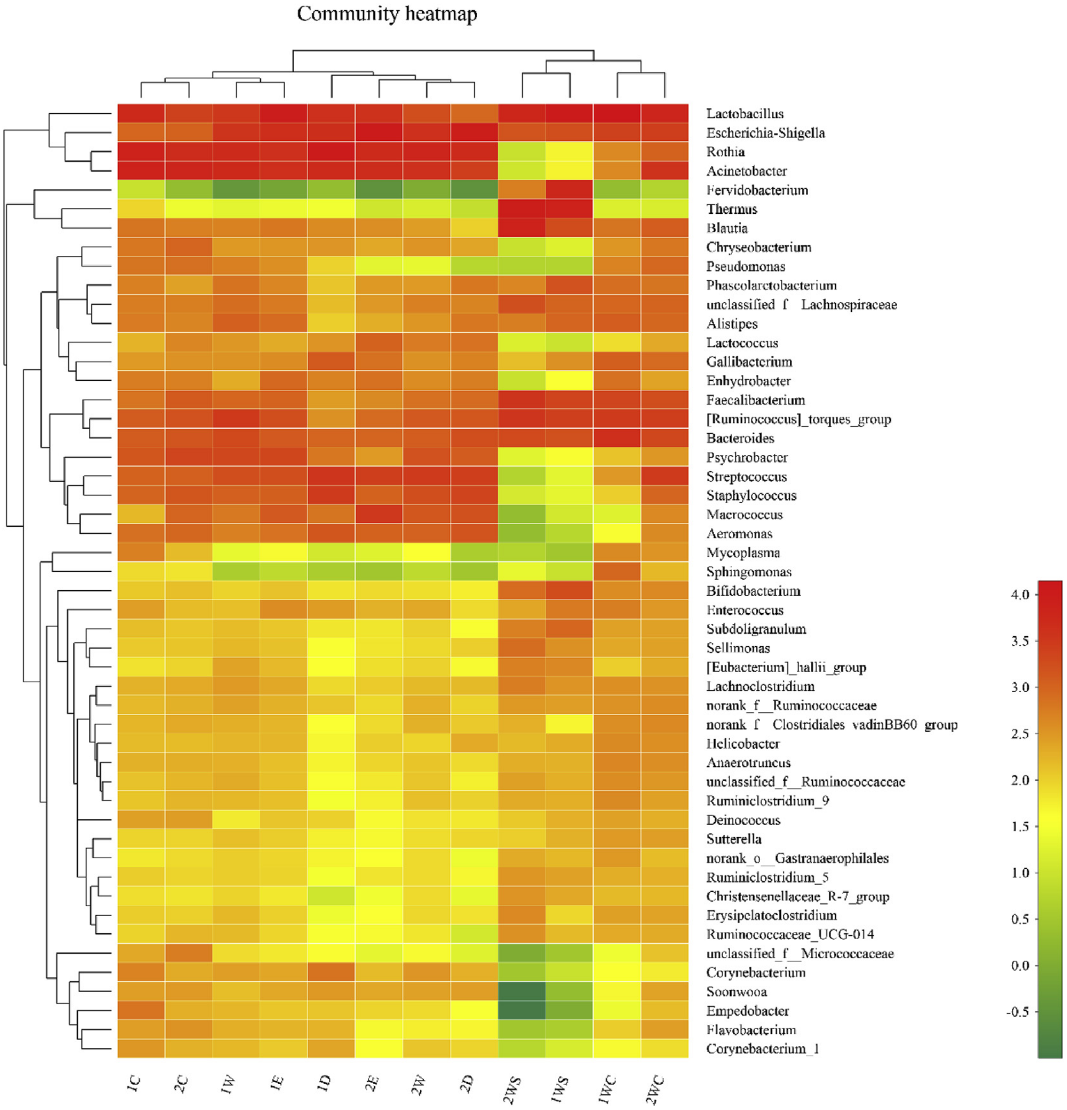

Fig. 6. Microbial community heatmap of the top 50 abundant genera (n = 5). Dendrograms of hierarchical cluster analysis grouping genera and samples are shown on the left and at the top, respectively. The color scale represents the normalized values of relative abundances by $\log_{10}$. Zero values were added as 1 and transformed by $\log_{10}$. D, carcasses after defeathering; E, carcasses after evisceration; W, carcasses after washing; C, carcasses after chilling; WS, processing water from the scalder tank; WC, processing water from the chiller tank; the numbers 1 and 2 before letters represent samples collected at the beginning and end of the processing day, respectively. Relative abundance is expressed as the mean value. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the scalder water. Thus, it is suggested that contamination from the scalder tank is not an important source of *Aeromonas* and *Streptococcus* on broiler carcasses.

The processing water used to immerse carcasses during the slaughtering process is a potential source of contamination (Goksoy et al., 2004; Guerin et al., 2010). *Proteobacteria* and *Firmicutes* represented over half of the sequences recovered from both the scalder tank and chiller tank during broiler processing (Rothrock et al., 2016). This finding was supported by the results of the bacterial composition of chiller tank water in the present study. In the scalder tank water samples, the predominant presence of *Firmicutes* was observed as expected; however, *Deinococcus-Thermus* was found to be the second most dominant phylum. The *Deinococcus-Thermus* phylum is known for its resistance to extreme stresses, including high temperature, radiation, oxidation and desiccation (Tian and Hua, 2010). Since scalding was performed on a hard-scald system (Buhr et al., 2014) with a water temperature of approximately 60 °C–63 °C, it was not surprising that the phylum *Deinococcus-Thermus* was found to be dominant in scalder tank water. Moreover, the thermophilic bacterium *Fervidobacterium,* which belongs to phylum *Thermotogae* (Otaki et al., 2012), was also detected in scalder tank water, supporting a report that scalding tends to be selective for heat-resistant mesophilic organisms (Goksoy et al., 2004). However, the abundance of *Deinococcus-Thermus* and *Thermotogae* on carcass samples was less than 1% of the total OTUs. The PCoA plot revealed that the bacterial profiles of carcass samples were more similar to chiller water samples than to scalder tank water samples (Fig. 4). The PCoA clustering results were confirmed by the Venn diagram analysis that showed that chilled carcasses shared a higher number of genera with water in the chiller tank than water in the scalder tank. Hence, the influence of scalding water on the bacterial communities of the surface of the carcasses (after chilling) was quite limited. We have identified one possible explanation, namely, the defeathering process, which occurred after the scalding process, removed the feathers of broilers, thereby providing a new surface that could be colonized by other bacteria during subsequent operations (Thomas and Mcmeekin, 1980; Allen et al., 2003).

Generally, the bacterial load of the equipment surfaces, contaminated processing water, and workers' hands are expected to increase or be maintained with increased processing time (Aburuwaida et al., 1994; Geornaras and von Holy, 2000). Zweifel et al. (2015) studied the microbiological contamination in three broiler abattoirs and found that the time of slaughter (morning, midday, or afternoon) had no consistent effect. In the current study, the aerobic mesophilic counts on carcasses after evisceration and chilling, as well as in the tank water, showed a significant increase with processing time (Fig. 2). The bacterial load of the scalder tank and chiller water would increase if the rate of fresh water addition were insufficient. In the present study, the time of slaughter had no consistent effect on taxonomic richness, evenness, and diversity of bacterial communities on broiler carcasses after chilling (Table 1). It is worth noting that richness, evenness, and diversity of bacterial communities in both scalder tank and chiller tank were relatively unchanged throughout the processing day. Similarly, one recent study carried out by Rothrock et al. (2016) showed that the bacterial richness in chiller tank water decreased over the processing day, while bacterial diversity and evenness were relatively unchanged. Moreover, the values of alpha diversity parameters of bacterial community in scalder tank plateaued halfway through the processing day (Rothrock et al., 2016). Therefore, it is plausible that immersed carcasses could introduce increased number of bacteria to the scalder tank and chiller tank throughout a processing day, without changing their bacterial diversity.

5. Conclusions

The present work provides a community-level investigation of the bacterial diversity of commercial yellow-feathered broilers during processing. The bacterial community structure on the broiler carcasses was observed to increase in complexity at later processing stages. Although immersion chilling showed a positive effect on reducing the bacterial load of the carcasses, the occurrence of cross-contamination during the chilling process was revealed. Compared to bacteria in scalder water, the bacterial diversity in the chiller water may play a more important role in the bacterial contamination of broiler carcasses. Since the differences in hygiene levels and slaughter process performance vary in different slaughterhouses, more targeted studies will be needed.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2019.04.006.

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