

Article

Evaluation of Bacterial Diversity and Quality Features of Traditional Sichuan Bacon from Different Geographical Region

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Featured Application: This work can help us understand the role of bacterial communities in Sichuan bacon more deeply and accurately.

Abstract: Sichuan bacon is one of the most popular types of Chinese bacon in the domestic market. High-throughput sequencing was used to characterize the bacterial diversity of 39 Sichuan bacon samples collected from 3 geographical regions. The results showed that the bacterial diversity of Sichuan bacon in different regions demonstrated certain specificity as well as similarity, and the shared OTUs were close to 81% of the total number in the basin group, mountain group, and plateau group. At the genus level, *Staphylococcus* is the most dominant genus among the three groups, covering 26.7%, 20.6%, and 22.7%, respectively. Beta diversity shows significant differences in bacterial compositions in different geographic regions, especially for *Pseudomonas*, *Brochothrix*, *Lactobacillus*, *Lactococcus*, and *Enterococcus*. Meanwhile, some physicochemical characteristics were analyzed, and a significant difference ($p < 0.05$) among the three regions was shown in the Aw, pH, and nitrite content, which were significantly correlated with undesired bacteria. This study provides insights into the understanding of the role of bacterial communities in the microbial safety and quality improvement of Sichuan bacon.

Keywords: Sichuan bacon; bacterial diversity; high-throughput sequencing; geographical regions; specificity; undesired bacteria



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1. Introduction

Chinese bacon, known as “larou” in China, is one of the most popular traditional dry-cured meats in the domestic market [1]. Southern China is an extremely suitable area for bacon production due to its geographical and climatic conditions, especially in the Sichuan province, which includes the basin plain (basin), the Qinghai-Tibet Plateau (plateau), and the mountainous transition zone (mountains), with altitude differences of more than 3000 m [2]. Traditional Sichuan bacon is produced in December on the lunar calendar. After slaughtering, the pork belly or hind leg meat is sampled and cut into uniform strips. The strips are evenly wiped with salt, layered in a ceramic tank, salted for 7–10 days, and turned over 2 or 3 times during this period. The meat strips are cleaned with warm water and air-dried naturally following the curing. Finally, all the strips are continuously hung outdoors or intermittently smoked by a cooking stove for ripening for about 3 weeks until becoming the final products. The entire production cycle is approximately 30–35 d. In Sichuan province, there are more than 2000 small- and medium-sized meat processing enterprises, and cured meat products such as Sichuan bacon and sausage account for 80% of the total meat product consumption. In the meantime, there are more than 10 million

families in Sichuan province, and each family will make Sichuan bacon in December on the lunar calendar every year. Sichuan bacon together with Jinhua ham, Cantonese sausage, and Nanjing dry-cured duck are the most famous traditional meat products in China.

In modern fermented meat production, the use of starter cultures, mainly constituted of lactic acid bacteria (LAB) and coagulase-negative staphylococci (CNS), is becoming more frequent to guarantee safety and to standardize product properties. For example, meat products with the addition of starter cultures have better flavor, quality, and safety properties than products without them [3,4]. Unfortunately, Sichuan bacon is still produced with traditional technologies and without selected starters, which means its production is more of an art, depending on the skill and experience of the meat manufacturer rather than a process fully based on scientific and technological means [5]. Although the use of commercially available starters may result in a loss of the desirable sensory characteristics found in artisan fermented meat with less flavor [6], industrialized production of starter cultures is a consequence of the gradual shift in traditional meat production from small, local producers to large-scale processing plants [7].

Previous studies showed that the most promising strains for starter cultures are those which are isolated from the indigenous microbiota of traditional products [8–10]. These strains are well adapted to the meat environment and the specific manufacturing process and thus are capable of dominating the microbiota of products [11]. Fermented meat products must be manufactured with the addition of autochthonous microorganisms not only for the preservation of regional typicality but also for avoiding risks to consumers' health [12]. In other words, the autochthonous microbiota of fermented meat products must be analyzed in detail, because commercial starter cultures usually originate either from substrates or from the processes in which they are applied [5]. In order to protect the traditional aspects of these products and select the autochthonous starter cultures to be used, it is essential to understand the microbial dynamics during fermentation [7].

However, the research on traditional Sichuan bacon has rarely been reported in the literature [13], and the bacterial community dynamics are still unknown. Although some strains have been studied intensively in laboratory patterns, the bacterial communities have yet to be completely clarified in fermented meat because of the drawback of the culture-dependent method [10]. Now, high-throughput sequencing (HTS) methods are particularly suitable for research of fermentation food due to the more accurate and in-depth information attained compared with traditional methods involving the microbiota, especially in spontaneous fermentation matrixes such as cheese, sourdough, kimchi, as well as cured meat products [14–17].

In this study, we identified the bacterial diversity of Sichuan bacon sampled from three altitude zones in Sichuan province of China by an HTS approach and distinguished the general features of bacons through its physicochemical characteristics. Our results are expected to provide a comprehensive comparison of the bacterial diversity profiles among three geographic regions and insights into the significant differences of the complex microbial compositions in Sichuan bacon of different areas. In the meantime, the undesired bacteria community was checked by HTS technology to evaluate the microbial safety of Sichuan bacon. These detailed data can help us understand the role of bacterial communities in Sichuan bacon more deeply and accurately.

2. Materials and Methods

2.1. Sample Collection

Thirty-nine samples of traditional Sichuan bacon were randomly collected from 27 individual households in 8 cities across the Sichuan province of China, which were classified into three groups: basin (a total of 14 samples, numbered BA1–BA4, BB1–BB5, and BC1–BC5), plateau (a total of 10 samples, numbered PA1–PA5, and PB1–PB5), and mountains (a total of 15 samples, numbered MA1–MA5, MB1–MB5, and MC1–MC5), according to their geographical origins (Table 1). All samples, with production cycles of 35 ± 2 d, were collected between 1 March 2018 and 5 March 2018. Each sample was vacuum-packaged in

situ and shipped back to the laboratory with ice packs. Each sample was minced and mixed after removing the visible skin, fat, and connective tissue in the ultra-clean workbench. Immediately, 10 g of minced samples were packed into a sterile homogenized bag for DNA sequencing. The remaining part of each sample was packaged for other tests and stored at -80°C .

Table 1. The information of the sampling locations.

Region	City	Encode	Average Temperature ($^{\circ}\text{C}$) ^A	Average Relative Humidity (%) ^A	Altitude (m)
Basin	Suining Leshan Chengdu	BA	7~8 $^{\circ}\text{C}$	75~80%	<500 m
		BB			
		BC			
Mountains	Yibin Ya'an Xichang	MA	9~10 $^{\circ}\text{C}$	75~80%	500~2000 m
		MB			
		MC			
Plateau	Kangding Ma'er kang	PA PB	−1~0 $^{\circ}\text{C}$	50~55%	>2500 m

All data come from the Sichuan Statistical Yearbook. ^A Results are expressed as a range of 5 years on average (2014–2018), and the data of each year only consist of December of the current year and January of the following year (the production period of Sichuan bacon).

2.2. Physiochemical Characteristics of Sichuan Bacon

The water activity (A_w) values were determined as previously described by Wang et al. [18]. One gram of minced sample was measured at 25°C using a water activity meter (HD-3A, Huake Instrument and Meter Co., td, Wuxi, China). The pH values were determined as described by Jin et al. [19] and were recorded using a pH meter (PHSJ-3F, INESA Scientific Instrument Co., Ltd., Shanghai, China). The salt content was scored as chloride and was quantified according to ISO 1841-1:1996(E). The peroxide value (POV) and nitrite content were measured as described by Jin et al. [20]. All experiments were performed in triplicate, and the results represented the mean values of three independent experiments.

2.3. DNA Extraction and Amplicon Sequencing

The total genome DNA from the samples was extracted using the CTAB/SDS method. The DNA concentration and purity were monitored on 1% agarose gels. The V4 region of the bacterial 16S rRNA gene was amplified with the universal primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The PCR mixture (30 μL) consisted of 15 μL of the Phusion[®] High-Fidelity PCR Master Mix (New England Biolabs), 0.2- μM primers, and 10 ng of template DNA. The amplification cycles were as follows: 98°C for 1 min; 30 cycles (98°C for 10 s, 50°C for 30 s, and 72°C for 60 s); and then a final extension at 72°C for 5 min. PCR products were mixed with the same volume of $1\times$ loading buffer (contained SYB green) and operated electrophoresis on 2% agarose gel for detection. Samples with bright main strips between 400–450 bp were chosen for further experiments. PCR products were mixed in equidensity ratios and then purified with a Qiagen Gel Extraction Kit (Qiagen, Hilden, Germany). Sequencing libraries were generated using a TruSeq[®] DNA PCR-Free Sample Preparation Kit (Illumina, San Diego, CA, USA) following the manufacturer's recommendations, and index codes were added. The library quality was assessed on a Qubit[®] 2.0 Fluorometer (Thermo Scientific, Waltham, MA, USA) and the Agilent Bioanalyzer 2100 system. At last, the library was sequenced on an IlluminaHiSeq2500 platform, and 250-bp paired-end reads were generated.

2.4. Sequence Analyses

Paired-end reads were assigned to samples based on their unique barcodes and truncated by cutting off the barcode and primer sequence. The paired-end reads were merged using FLASH (V1.2.7) to obtain raw tags. Then, quality filtering of the raw tags

was performed under specific filtering conditions to obtain the high-quality clean tags according to the QIIME (V1.7.0) quality-controlled process. After chimeric sequences were identified and removed by the UCHIME algorithm (UCHIME Algorithm), effective tags were obtained and clustered into operational taxonomic units (OTUs) with a 97% similarity threshold. Taxonomy assignment of the OTUs was performed by comparing the sequences to the SILVA database.

The abundance information of the OTUs was normalized by the sample with the least sequences. A Venn diagram was illustrated to display the similarities and differences of the communities among the regions. Alpha diversity (Chao1 and ACE richness estimators, Shannon and Simpson diversity indices, and Good's coverage) was used to analyze the bacterial diversity in a single sample. The beta diversity based on the Bray-Curtis and Unifrac distance was used to evaluate the differences between the microbial communities. Principal component analysis (PCA), principal coordinate analysis (PCoA), and non-metric multidimensional scaling (NMDS) were performed using QIIME software (Version 1.7.0). In the meantime, linear discriminant analysis (LDA) effect size (LEfSe) was applied to illustrate statistically significant biomarkers in each region with $LDA > 4$ [21].

2.5. Statistical Analysis

The collected data were subjected to one-way analysis of variance (ANOVA). Statistical significance in the group means was evaluated at $p < 0.05$ using Duncan's multiple range tests. Statistical analysis of the data was carried out in SPSS 22.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Physicochemical Parameters of Sichuan Bacon

The results for the water activity (A_w), pH, salt content, nitrite content, and peroxide value (POV) of all samples from three different regions are shown in Table 2. The average values of the POV and salt content of three groups were consistent with previous reports [19], and no significant difference ($p \geq 0.05$) was observed among the groups. However, the POV and salt content of the basin group bacons were the highest, and those of the plateau group bacons were the lowest among the three groups. This phenomenon could be due to the fact that NaCl had a pro-oxidant effect on the lipids in cured meat products within the certain design range, especially for lipid primary oxidation [22].

The average A_w values of bacons from the mountains group and plateau group were 0.855 and 0.879, respectively, with no significant difference ($p \geq 0.05$). Conversely, the average A_w value of the basin group (mean = 0.814) was significantly ($p < 0.05$) lower than that of the other two groups. All samples were collected from different locations within only 7 days and were produced by similar processing times (about 35 d). However, dehydration of bacon in the basin group was more severe than for the other two groups. The reason for this might be that the free water of the samples evaporated more easily into the air in the basin region than in the other two regions during bacon processing. Another possible explanation might be that there were some slight differences in the production process of Sichuan bacon in different regions. As with the A_w , the average pH value of the basin group was significantly ($p < 0.05$) lower than that of the mountain and plateau groups. The metabolism of both the acid-producing bacteria in the initial curing period and the ammonia-producing bacteria in the later ripening period led to changing the pH of the meat matrix and further affecting the pH of the final product [23]. The average pH values of the basin, mountain, and plateau groups were 5.45, 5.68, and 5.92, respectively, which might indicate that there were some differences in the metabolic activity of those bacteria in the samples from the three regions.

Although no nitrite was added to the traditional Sichuan bacon production, a low content of nitrite in all samples was observed. Interestingly, the nitrite content of the mountains group was significantly ($p < 0.05$) higher than those of the basin and plateau groups. In the production of cured meat products, nitrate could be reduced to nitrite by

nitrate reductase from microorganisms such as *Staphylococcus* and then to nitric oxide (NO), which contributes to color development and microbial safety [24].

Table 2. Physicochemical indices in Sichuan bacon subjected to three groups of different regions.

Parameters	Groups		
	Basin (<i>n</i> = 14)	Mountains (<i>n</i> = 15)	Plateau (<i>n</i> = 10)
Aw			
Means ± SE	0.814 ± 0.037 ^b	0.855 ± 0.023 ^a	0.879 ± 0.049 ^a
Range	0.761~0.924	0.825~0.912	0.828~0.974
pH			
Means ± SE	5.45 ± 0.36 ^b	5.68 ± 0.30 ^{ab}	5.92 ± 0.25 ^a
Range	5.02~6.03	5.27~6.48	5.59~6.44
Salt content^A			
Means ± SE	3.37 ± 1.19	3.00 ± 0.86	2.807 ± 1.359
Range	1.88~5.24	1.87~4.95	1.01~5.53
Nitrite content^B			
Means ± SE	1.12 ± 0.80 ^b	4.80 ± 3.46 ^a	2.57 ± 2.15 ^b
Range	0.21~2.39	0.56~13.34	0.35~8.06
Peroxide value^C			
Means ± SE	17.73 ± 17.22	13.59 ± 9.29	13.51 ± 6.70
Range	1.54~61.70	3.40~34.00	7.59~26.58

Results are expressed as means with standard errors including 14, 15, and 10 samples, respectively, and each sample data were means of three replicates. ^A Expressed as g/100 g muscle. ^B Expressed as mg/kg dry basis. ^C Expressed as meq O₂/kg of fat. ^{a,b} Means in the same row with different superscripts differ significantly (*p* < 0.05).

3.2. Characteristics of the Sequencing Data

As a result of quality control, an average of 77,311 high-quality sequences were obtained from each bacon sample (Table A1), which agreed with previous works in which the number of reads about each fermentation meat ranged from 33,983 to 170,428 [25]. All sequences were assembled to 1364 OTUs based on a 97% identity level. The indices of alpha diversity are shown in Table A2. Sufficient coverage of data was proven by up to 99% of the Good's coverage for all the samples. Although the amount of OTUs in each sample fluctuated obviously, ranging from 53 to 769, there was no difference between the basin, mountains, and plateau groups, owing to the total OTUs that were clustered to 1156, 1360, and 1298, respectively. In the meantime, the amount of shared OTUs among the three regions was up to 1101 (Figure 1), close to 81% of the total number of OTUs, indicating a high level of similarity in the bacteria compositions among the three groups. Traditional Sichuan bacon has developed its own style through long-term development, and the key formulations and manufacturing processes have remained almost unchanged since the Ming Dynasty, although a wide variety of bacons come from the food preferences of the different regions in Sichuan. This may have been the reason why the bacterial diversity of bacon in different regions was similar.

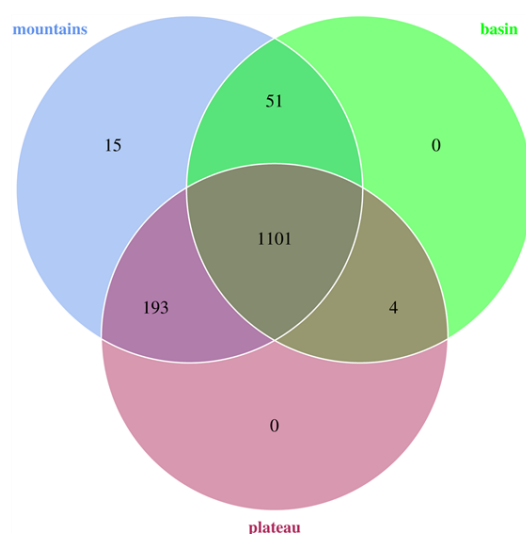


Figure 1. Venn diagrams of the three regions based on OTUs (3% distance). Basin: 14 bacons collected from Suining, Leshan, and Chengdu; mountains: 15 bacons collected from Yibin, Ya'an, and Xichang; plateau: 10 bacons collected from Kangding and Ma'er kang.

3.3. Bacterial Compositions of Sichuan Bacons

The relative abundances (%) of bacterial community proportions at the phylum and genus level are shown Figure 2. Firmicutes and Proteobacteria were the dominant phyla in all samples, ranging from 77.1% to 98.9%, and most samples revealed levels above 90%. Apart from these two phyla, Actinobacteria, Oxyphotobacteria, and Bacteroidetes were observed in some samples at levels above 1% (Figure 2A). This agrees with research on other Chinese traditional meat products such as Chinese sausage and ham [18,26]. At the genus level (Figure 2B), *Staphylococcus* was the dominant genus in the basin, mountains, and plateau group bacons, covering 26.7%, 20.6%, and 22.7% of the total genus, respectively. Conversely, the relative abundances of *Lactobacillus*, accounting for 0.5%, 3.5%, and 2.5%, respectively, were much lower than those of *Staphylococcus*. Many researchers support the belief that LAB and CNS are the most active microorganisms derived from meat fermentation [5]. The previous studies focused more on *Lactobacillus* diversity and function in Chinese traditional meat products [27], while little attention was paid to *Staphylococcus*. Moreover, the diversity of CNS in meat products is complex and variable because it is subject to multiple factors, including the type of raw muscle used, the ingredients added, and the processing conditions applied [28]. For instance, the pH increases due to the use of lactate during post-ripening, leading to replacement of the dominant species from *S. saprophyticus* to *S. equorum*, which is more adapted to weaker acidic environments [29]. It is reasonably believed that the role of *Staphylococcus* in the formation of the unique flavor and quality of Sichuan bacon should be paid more attention in future research.

In addition to *Staphylococcus*, some other predominant genera of relative abundance were observed in the samples. For instance, *Phyllobacterium* (16.8%), *Psychrobacter* (11.5%), *Brochothrix* (6.5%), *Vibrio* (6.3%), and *Delftia* (3.2%) were the abundant genera in the basin group, which are commonly spoilage bacteria in meat products [30]. In the plateau group, the relative abundance of *Pseudomonas* was up to 10.2%, which was much higher than the other two groups. This gram-negative bacteria, which relied on proteolytic activity, was a predominant spoilage bacteria in stored meat under low-temperature and aerobic conditions [31]. In the meantime, *Brucella* (5.1%), unidentified *Clostridiales* (3.7%), *Vibrio* (3.4%), *Lactococcus* (3.4%), and *Enterococcus* (3.2%) were observed in the plateau group, and *Phyllobacterium* (8.0%), *Lactobacillus* (3.5%), *Pseudomonas* (3.4%), and *Brucella* (3.1%) were observed in the mountains group. Undesirably, unidentified *Rickettsiales* (21%), which has never been found in bacon, was one of the dominant genera in the mountains group. The genus was derived from raw meat contamination, owing to this prokaryote only

parasitizing eukaryotic cells [32]. Although spontaneous fermented meat products are recognized as having excellent flavor, this can sometimes give rise to poor quality products, including decreased appearance quality, a shortened shelf life, and increased health risks. For this reason, the addition of starter cultures in traditional meat products is the best way to reduce potential risk caused by microorganisms and is also the best choice for Sichuan bacon.

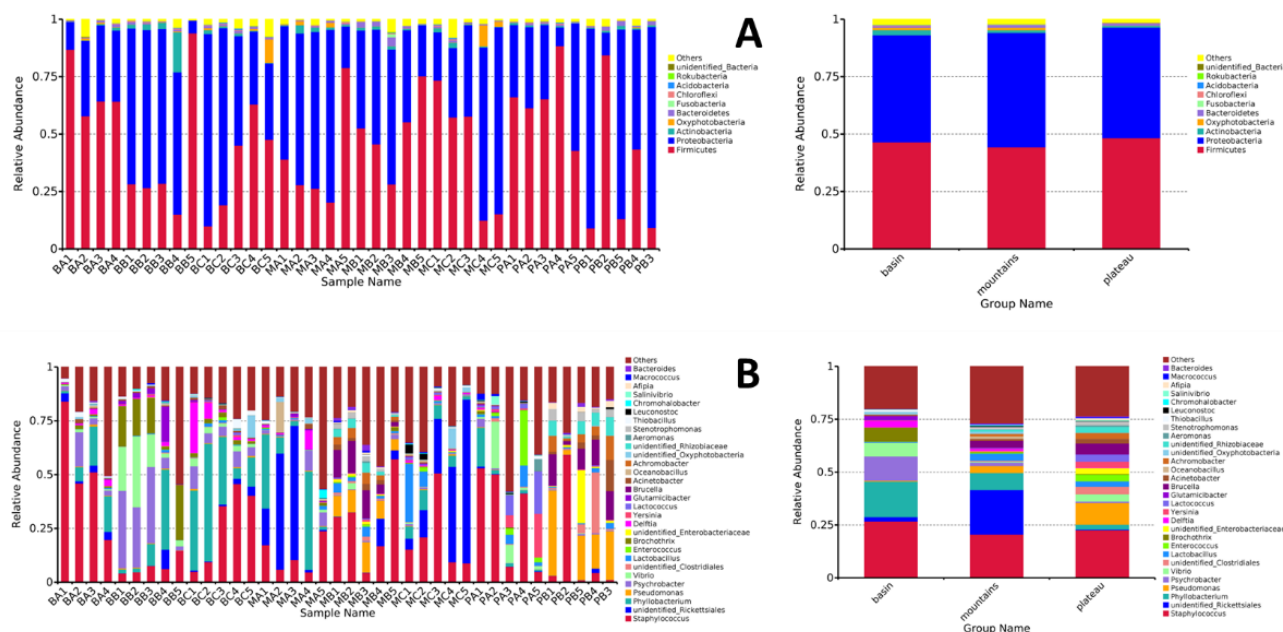


Figure 2. The relative abundances of bacterial compositions of different bacons (A) at the phylum level and (B) at the genus level.

3.4. Analysis of Bacterial Communities of Sichuan Bacon in Different Geographical Regions

The differences between the bacterial communities was compared in the samples collected from three regions of Sichuan to investigate the association between sampling location and bacterial communities (Figure 3). For PCA analysis (Figure 3A), there were 22.36% and 13.23% total variations in the first and the second components, respectively. PCA analysis showed that the samples collected from the basin region could be clustered together in small ranges. Interestingly, only a part of the mountains and plateau samples were clustered with the basin group, and the remaining samples were scattered long distance. PCoA (PC1 58.43% and PC2 15.32%, Figure 3B) and NMDS (stress: 0.081, Figure 3C) analysis based on the Weighted Unifrac distance showed a similar clustering pattern in the bacterial structures for the three groups. Moreover, a significant test of the community structure difference between groups based on the Anosim nonparametric test method of OTU relative abundance showed that there was a significant difference ($p < 0.05$, data not shown) between the basin and mountains, basin and plateau, mountains and plateau, or basin, mountains, and plateau. Considered together, the bacterial diversity of Sichuan bacon in different geographical regions showed a certain specificity as well as similarity. It is believed that long-term regular processes in spontaneous fermentation food often leads to a stable microbiota, among which only the best-adapted strains will occur [33]. On the contrary, microbial communities are strongly influenced by external environment conditions, such as a low environment temperature due to a production date from December of the current year to January of the following year, a high level of salt stresses due to formulation, or low water activity due to the long production cycle in meat ripening.

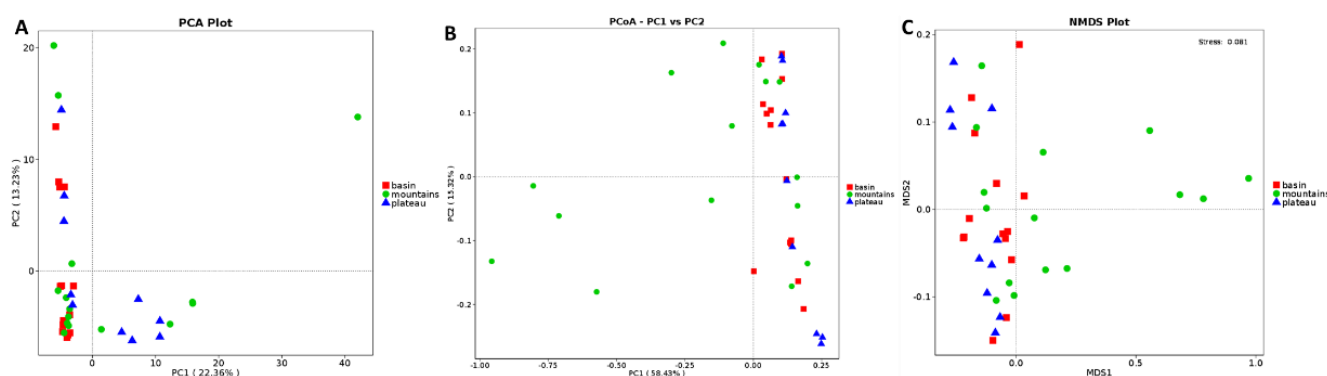


Figure 3. The beta diversity of the 39 bacons at the genus level (PCA (A), PCoA (B), and NMDS (C) scores plotted based on Weighted Unifrac).

LefSe analysis was able to identify statistically significant biomarkers, which were bacterial taxa about significant differences between the groups. The taxon nodes were displayed in terms of LDA scores >4 (Figure A1), and a few predominant genera are illustrated in Figure 4. The relative abundances of *Lactobacillus* in the mountains and plateau groups were much higher than those in the basin group (Figure 4A), while the opposite was true for *Staphylococcus*. The results showed that there was a negative correlation in the relative abundance between *Lactobacillus* and *Staphylococcus*, which is perhaps one of the most important factors in shaping the typical style of Sichuan bacon. Interestingly, the relative abundances of *Lactococcus* in the plateau group (Figure 4B), similar to *Enterococcus* (not listed), were much higher than those in the basin and mountains groups. These genera also can produce a large amount of organic acid, such as acetic and lactic acid, during exponential growth, following conversion to the alcohols and ketone that contribute a richer flavor and aroma to the product [34,35]. It is suggested that *Lactococcus* and *Enterococcus* are the predominant acid-producing bacteria rather than *Lactobacillus* in the plateau group. Moreover, the relative abundances of *Brochothrix* in the basin group were much higher than those in the mountains and plateau groups (Figure 4C), while the relative abundances of *Pseudomonas* from the basin group were much lower than those from the mountains and plateau groups (Figure 4D). Although there were undesirable microorganisms present in all samples of Sichuan bacon due to outdoor ripening processing, the undesirable microflora was different between regions, which means that different strategies should be prepared to ensure the safety of the products.

3.5. The Relationship between Top Genera and Environmental Factors

The commonly accepted view is that variables such as the Aw, pH, salt content, POV, and nitrite can induce changes in microbial communities in microbial ecosystems [36]. Five environmental factors were screened using VIF and BioENV tools, and the results demonstrated that the environmental factors had a significant impact on the microbiota. Parametric correspondence analysis (CCA, Figure 5A) and Spearman analysis (Figure 5B) were used to further investigate the relationship between the top genera (top 15 genera of relative abundance) and environmental factors. Obviously, the salt content and peroxide value had no significant ($p > 0.05$) effect on the bacterial community, owing to no significant difference between the three groups in this test. Aw was found to be positively correlated with the relative abundances of *Lactobacillus* and negatively correlated with the relative abundances of *Brochothrix*, *Vibrio*, *Delftia*, and *Psychrobacter*. The pH was negatively correlated with the relative abundances of *Delftia*, *Phyllobacterium*, and *Psychrobacter*. Nitrite was positively correlated with the relative abundances of unidentified Clostridiales, *Brucella*, *Lactococcus*, *Pseudomonas*, and unidentified enterobacteriaceae and negatively correlated with the relative abundances of *Psychrobacter*, *Brochothrix*, *Vibrio*, *Phyllobacterium*, and *Delftia*. The results showed that nitrite was the most important factor due to significant correlation

with many critical genera, followed by the Aw and pH. Nitrite plays a decisive role in cured meat products, providing a high-quality red color and flavor for products, especially for being recognized as bacteriostatic and bactericidal agents against pathogenic bacteria [37]. Nitrite that considered harmful to human health is not added in traditional Sichuan bacon, however its integrated functions are still difficult to replace in the meat industrialization. A combined strategy may be a better solution than a single agent. Interestingly, all three environmental factors were mostly negatively correlated with the undesired microorganisms in Sichuan bacon, which revealed that an appropriate level of Aw, pH, and nitrite plays a vital role in the safety of Sichuan bacons.

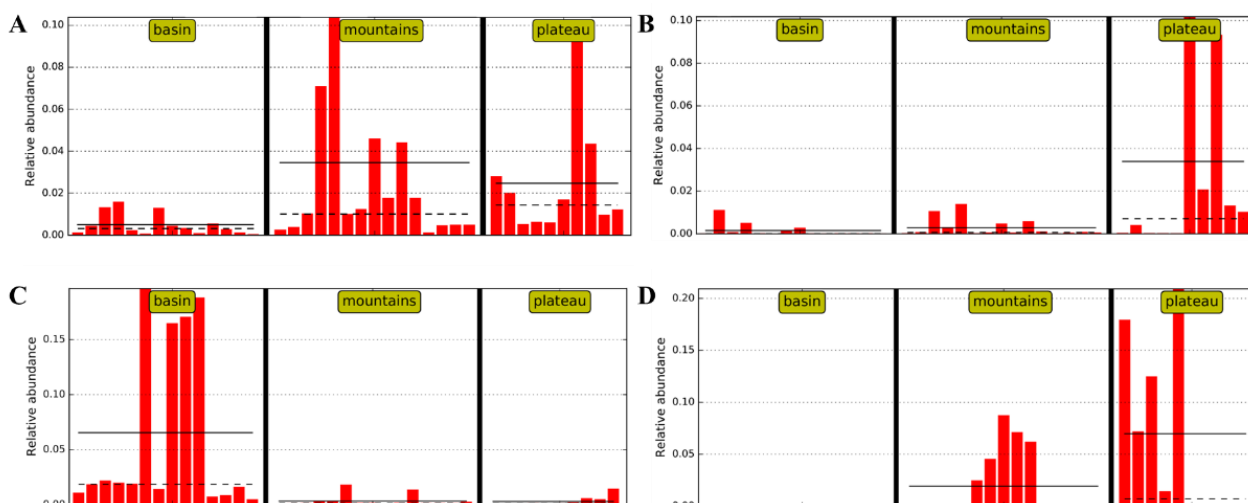


Figure 4. Significantly different genera through LeSe analysis of different abundant taxa among three regions of Sichuan bacon: (A) *Lactobacillus*, (B) *Lactococcus*, (C) *Brochothrix*, and (D) *Pseudomonas*.

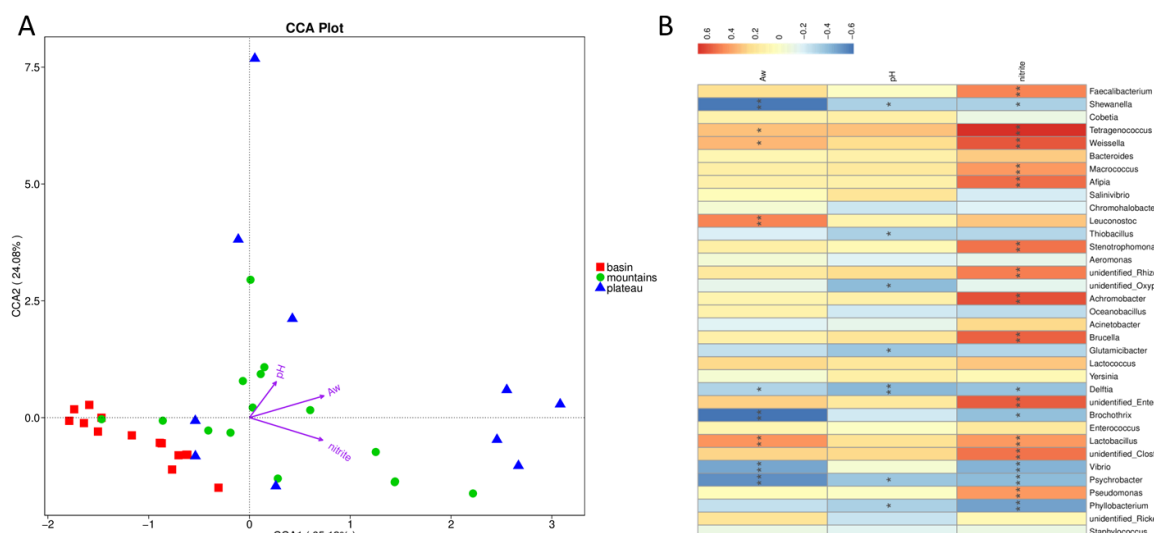


Figure 5. The parametric correspondence analysis (A) and Spearman analysis (B) of the top genera and environmental factors among the three regions.

4. Conclusions

In conclusion, there was a large number of undesired microorganisms in Sichuan bacon collected from three geographical regions, which was significantly correlated with the physiochemical characteristics. Our data suggested that *Staphylococcus* played the most important role in Sichuan bacon, and *Lactococcus* and *Enterococcus* were more important in the plateau group than in the other two groups. There were significant differences in

undesired bacterial community structures among the geographical groups, which were also affected by physiochemical parameters such as the Aw, pH, and nitrite levels. This may indicate that a variety of control strategies should be applied to Sichuan bacon in different geographical regions. Furthermore, more studies regarding the species and strain levels of bacterial diversity in Sichuan bacon by metagenomics and culturomics are needed. Moreover, fermentation starters consisting of functional strains to improve the safety and quality of industrial products are also necessary.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. The number of sequences for each sample.

Sample	Raw PE (#)	Combined (#)	Qualified (#)	Nochime (#)	Avg Len (nt)	Effective %
BA1	98,835	96,773	95,663	93,267	252	94.37
BA2	87,792	63,104	60,343	57,774	254	65.81
BA3	86,549	84,950	83,974	80,927	252	93.5
BA4	83,900	79,274	77,945	75,347	252	89.81
BB1	84,898	82,385	81,136	73,781	253	86.91
BB2	86,495	83,717	82,557	76,913	253	88.92
BB3	95,010	90,558	89,135	84,040	253	88.45
BB4	92,873	83,191	81,768	76,352	249	82.21
BB5	87,069	75,142	73,213	70,254	254	80.69
BC1	81,467	73,264	70,411	66,509	252	81.64
BC2	98,199	94,404	93,045	87,076	253	88.67
BC3	85,961	76,897	75,270	71,158	253	82.78
BC4	94,842	80,522	77,919	72,685	253	76.64
BC5	95,153	76,999	74,828	71,166	252	74.79
MA1	82,378	76,064	74,786	72,287	249	87.75
MA2	85,426	83,136	82,413	79,576	237	93.15
MA3	97,461	88,505	87,206	85,441	234	87.67
MA4	83,188	82,167	81,227	76,744	253	92.25
MA5	92,712	87,305	85,919	82,609	253	89.1
MB1	82,432	79,911	79,157	73,762	254	89.48
MB2	89,052	84,857	83,635	79,450	254	89.22
MB3	76,775	73,559	71,681	66,512	255	86.63
MB4	88,555	85,598	83,973	80,118	250	90.47
MB5	93,716	91,280	90,427	87,884	252	93.78
MC1	92,442	87,084	85,654	79,627	252	86.14
MC2	98,422	82,567	80,554	76,418	250	77.64
MC3	84,825	80,792	79,890	77,608	245	91.49
MC4	99,950	95,372	94,375	91,363	239	91.41

Table A1. Cont.

Sample	Raw PE (#)	Combined (#)	Qualified (#)	Nochime (#)	Avg Len (nt)	Effective %
MC5	90,170	88,416	87,902	86,234	229	95.63
PA1	89,505	85,058	83,807	80,253	253	89.66
PA2	96,874	88,771	86,815	81,797	253	84.44
PA3	98,694	95,418	93,892	86,847	253	88
PA4	86,633	82,189	80,884	76,942	253	88.81
PA5	87,876	70,756	68,491	65,280	254	74.29
PB1	80,545	77,321	76,581	74,041	253	91.93
PB2	77,140	74,767	72,543	69,946	254	90.67
PB3	88,085	84,479	82,851	77,546	253	88.04
PB4	88,531	86,156	84,593	80,461	253	90.88
PB5	76,757	74,594	73,210	69,141	254	90.08

All data come from the report of NOVOGENE. The (#) represents the number of sequences and the (nt) represents average base-pairs number of effective data.

Table A2. Alpha diversity indices of each sample.

Sample	Observed Species	Shannon	Simpson	Chao1	Ace	Good's Coverage
BA1	346	1.5303	0.3083	357.413	368.0392	0.9991
BA2	281	3.2806	0.7633	277.4737	279.2758	0.9998
BA3	357	2.9900	0.7031	358.4685	385.1882	0.9993
BA4	448	3.1576	0.7523	463.6195	475.4967	0.9994
BB1	306	3.6041	0.8341	312.2462	334.4232	0.9996
BB2	349	3.4318	0.8120	362.2581	367.6659	0.9994
BB3	389	3.6992	0.8556	400.7222	419.8041	0.9993
BB4	749	4.1670	0.8242	755.6667	769.4436	0.9988
BB5	53	1.8340	0.6303	52.88235	58.51154	0.9999
BC1	680	3.8438	0.7982	696.4286	708.2509	0.9991
BC2	683	3.3852	0.6842	683.7653	710.7533	0.9991
BC3	706	3.7436	0.7757	713.7213	725.2032	0.9993
BC4	557	3.8612	0.7721	565.3404	577.2322	0.9993
BC5	534	3.6825	0.8016	562.6241	567.1605	0.9989
MA1	284	3.0673	0.7926	294.9014	294.3744	0.9995
MA2	312	3.6688	0.8350	321.0851	327.6277	0.9993
MA3	377	2.8811	0.6826	379.6726	375.6161	0.9994
MA4	511	3.1646	0.7491	513.4286	540.6371	0.9992
MA5	374	3.1296	0.7168	372.0435	387.8292	0.9994
MB1	610	4.8808	0.8810	608.28	633.0211	0.9993
MB2	594	4.5379	0.8608	609.1866	612.6939	0.9995
MB3	740	6.3633	0.9614	740.6842	740.852	0.9996
MB4	617	4.5869	0.8680	627.4681	640.6145	0.9994
MB5	476	3.1935	0.6678	501.5	497.8253	0.9989
MC1	706	4.3571	0.8640	705.52	725.3433	0.9992
MC2	769	4.5736	0.8899	772.4904	792.9436	0.9991
MC3	464	3.1463	0.7101	475.0074	485.7014	0.9992
MC4	470	3.5864	0.8186	498.1161	513.0337	0.9986
MC5	368	2.2450	0.5116	386.7835	419.8829	0.9991
PA1	363	3.0520	0.6912	362.2222	375.9001	0.9995
PA2	646	3.2985	0.7065	642.3623	664.7128	0.9991
PA3	544	3.8095	0.8344	571.2261	579.1615	0.9990
PA4	646	3.3790	0.7589	637.9827	669.531	0.9992
PA5	382	3.7926	0.8719	398.703	414.029	0.9992
PB1	544	4.1810	0.8650	544.175	563.6767	0.9993
PB2	416	2.6434	0.6151	436.2581	435.0155	0.9993
PB3	539	4.5015	0.9082	554.5118	559.1409	0.9992

Table A2. Cont.

Sample	Observed Species	Shannon	Simpson	Chao1	Ace	Good's Coverage
PB4	522	4.4449	0.8858	533.8261	533.6284	0.9994
PB5	474	4.6674	0.9015	491.8583	493.4462	0.9993

All data come from the report of NOVOGENE.

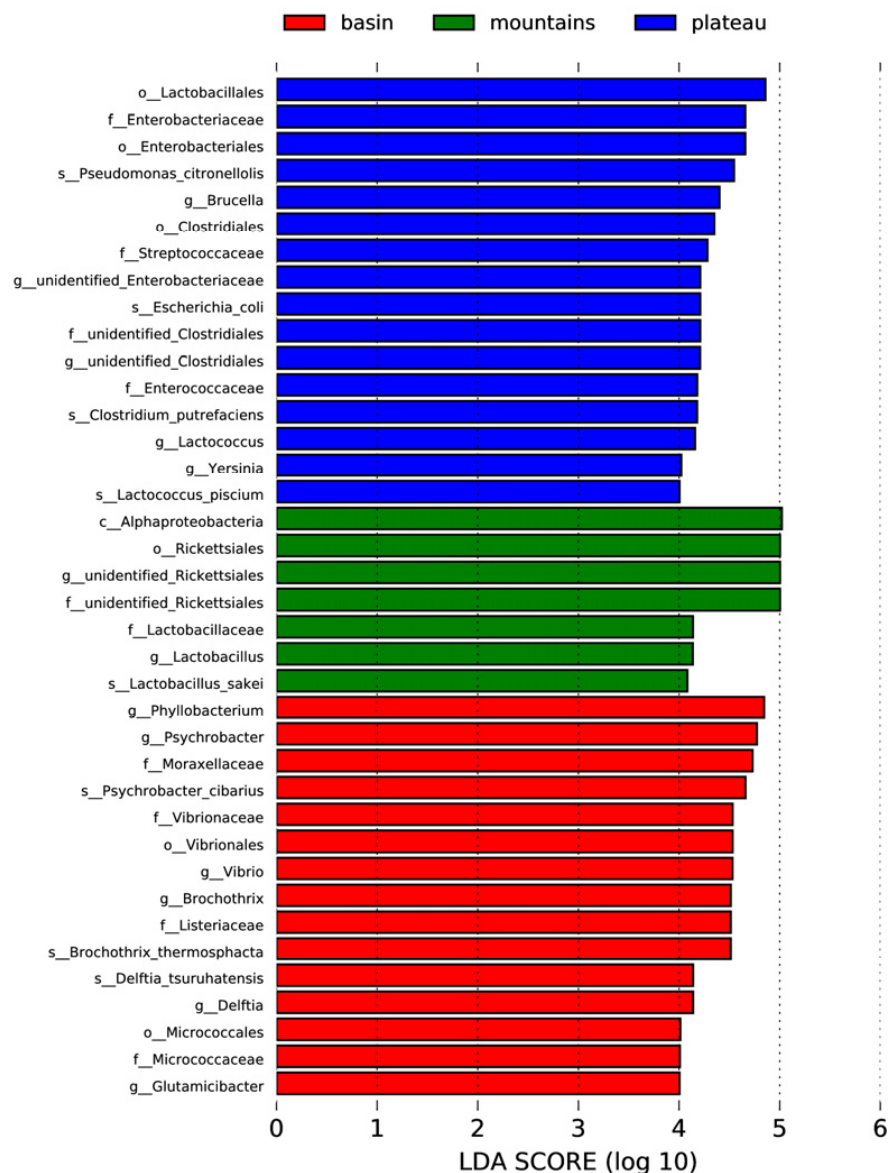


Figure A1. Significantly discriminant taxa (LDA score >4) among three regions of Sichuan bacon by LefSe analysis.

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