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Changes in the microbial communities in vacuum-packaged smoked bacon during storage

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ABSTRACT

This study aimed to gain deeper insights into the microbiota composition and population dynamics, monitor the dominant bacterial populations and identify the specific spoilage microorganisms (SSOs) of vacuum-packed bacon during refrigerated storage using both culture-independent and dependent methods. High-throughout sequencing (HTS) showed that the microbial composition changed greatly with the prolongation of storage time. The diversity of microbiota was abundant at the initial stage then experienced a continuous decrease. Lactic acid bacteria (LAB) mainly *Leuconostoc and Lactobacillus* dominated the microbial population after seven days of storage. A total of 26 isolates were identified from different growth media using traditional cultivation isolation and identification method. *Leuconostoc mesenteroides* and *Leuconostoc carnosum* were the most prevalent species since day 15, while *Lactobacillus sakei* and *Lactobacillus curvatus* were only found on day 45, suggesting that they could be responsible for the spoilage of bacon. *Serratia, Rahnella, Fusobacterium* and *Lactococcus* underwent a dramatic increase at some point in individual batchs which may be considered as potential contributors to the spoilage.

1. Introduction

Food spoilage is a great economical problem which is caused by the growth and metabolism of specific microorganisms (Huis in't Veld, 1996). Packaged meat products of animal' origin are susceptible to natural contamination caused by various bacteria (Borch et al., 1996; Casaburi et al., 2015; Chaillou et al., 2015; Korkeala and Björkroth, 1997; Samelis et al., 2000a). The shelf-life of packed meat products ranging from several days to several weeks. Bacon is a cured meat product produced mainly from pork belly which is widely consumed around the world (Soladoye et al., 2015). The consumption of cooked meat products especially smoked bacon is steadily increasing in China. The higher moisture content, lower salt, near-neutral pH and availability of nutrients in most modern bacon (Hughes, 1988; Sheard, 2010; Sheard et al., 2001) facilitated microbial growth, which led to the spoilage. The spoilage can be considered the result of microbial activity of a wide variety of microorganisms, indicated by off-flavors, off-odors, discoloration, slime, decrease in pH or unacceptable to the consumer before the sell-by date (Borch et al., 1996; Gram et al., 2002; Nychas et al., 2008). The bacon quality, including microbial quality, physicochemical attributes (such as flavour, colour, appearance, TVB-N, pH and fatty acid composition) and palatability (such as saltiness and crispiness) (Person et al., 2005; Soladoye et al., 2015). TVB-N, pH-value and colour changes have been suggested as chemical indicators of microbial spoilage in meat and meat products (Borch et al., 1996; Huang et al., 2014; Metaxopoulos et al., 2002). In recent years, microbial spoilage has become a major concern for the bacon industry in China. It is necessary to investigate the dynamics of contaminating bacteria and predominant bacterium species during storage.

Culture-dependent traditional microbial cultivation and characterization methods have been extensively used to identify the microorganisms in meat and meat products (Barakat et al., 2000; Björkroth et al., 1998; Metaxopoulos et al., 2002; Samelis et al., 2000a). A great diversity of bacteria can be found in different types of cooked meat products include *Lactobacillus sakei*, *Lactobacillus curvatus*, *Lactobacillus alimentarius*, *Weissella viridescens*, *leuconostocs*, *Enterobacteriaceae*, *Brochothrix thermosphacta*, yeasts and moulds (Borch et al., 1996; Cayré et al., 2005; Korkeala and Björkroth, 1997; Samelis et al., 2000a, 2000b). However, only 0.1–3% bacteria can be cultivated which are unable to represent the whole microbiota (Amann et al., 1995; Cocolin

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et al., 2013). Now, one of the main challenges for microbiologists is to develop strategies to understand the microbial physiology and its control. Although the culture-dependent method has some limitations, it is still an increasing interest due to its versatility (Alain and Querellou, 2009).

Culture-independent methods are more advanced and reliable when analyzing microbial communities. Polymerase Chain Reaction (PCR) cloning and the sequencing of 16S ribosomal RNA gene (rRNA) are frequently applied as the standard methods for the cultivation-independent survey (Klindworth et al., 2013). After decades of development, fingerprinting molecular methods and PCR-denaturing gradient gel electrophoresis (PCR-DGGE) have been frequently employed to study the microbiota changes within the shelf-lives of meat and meat products (Cocolin et al., 2013); however, small fragment size, low resolution power, background disturbance and difficulties in quantitative analysis hindered the use of PCR-DGGE (Polka et al., 2015). In comparison, HTS offers a more advanced method which assists scientists' assessment and understanding of microbial communities, and it provides a comprehensive description of the complex interactions among the species (Polka et al., 2015). This is because it can generate far more reads than traditional culture-independent methods and facilitate the discovery of more microbiota diversity as well as the detection of bacteria involved in high or low quantities of unknown origin (Ercolini, 2013). HTS is more efficient for monitoring the evolution of microbiota of meat and meat products within their shelf-lives. As evidence, 454 pyrosequencing had been successfully applied in Zhenjiang Yao meat during refrigerated storage by Xiao et al. (2013). Although the entire microbial diversity can be revealed by HTS, identification of dominant spoilage species cannot be obtained. Concerning the dominant species from spoilage microorganisms by more scientists, the purpose to further study spoilage potential and exploit controlling methods species of spoilage microorganisms. Compared to HTS, the traditional approach provides scientists a better method for isolation and identification of the specific species inducing meat spoilage (Casaburi et al., 2011). The specific species or strains can be obtained by culture-dependent methods (microbial cultivation) for the in-depth study of physiology, genetics and measures of industrial application (Alain and Querellou, 2009; Prakash et al., 2013). Culture-independent must be improved by accompanying developments arising from research using microbial cultivation. For instance, the microbiota of typical Italian salami was investigated by HTS, PCR-DGGE and culture-based methods, where *Staphylococcus* and *Lactobacillus* were identified as the main species during ripening stages (Polka et al., 2015). Tian et al. (2017) paired culture-dependent and HTS techniques to analyze the microbiological ecology of cooked pork during refrigeration. The results shows that the diversity of microbiota is negatively related to storage time, and the most prevalent genera in the initial stage (first two weeks) contained *Bacillus*, *Pseudomonas*, *Enterococcus* and *Lactococcus* and were replaced by *Carnobacterium* later in the storage period. The application of this combined technology in vacuum-packed lamb was also successful which *Carnobacterium*, *Yersinia* and *Clostridium* spp. were considered as the SSOs at −1.2 °C, while at 8 °C the SSOs expanded to include *Hafnia*, *Lactococcus* and *Providencia* spp. (Kaur et al., 2017).

However, to the best of our knowledge, the microbial community of smoked bacon during storage has not been studied by a combination of HTS and traditional cultivation methods. Therefore, a combined approach could be an appropriate strategy with which to investigate bacterial diversity and characterize the SSOs in bacon for exploiting appropriate controlling methods.

2. Materials and methods

2.1. Sample collection and experimental work

Samples of bacon were manufactured in joint venture-factories of China and Italy (Ma'anshan yurun, Anhui, China). To analyze the spoilage biota at different storage periods, five batches were prepared at different times and numbered 1 to 5. Samples were quickly transported to the laboratory with Drikold and then stored at 0–4 °C. The fresh smoked bacon (0 day) were analysed at vacuum-packaged and then at 7, 15, 22, 30, and 45 days during storage. Samples of each batch were refrigerated and stored for testing in triplicate at each time point.

2.2. Physicochemical analysis

2.2.1. pH

Sample (10 g) was harvested from the triplicates samples which were minced and mixed at each sampling point. They were then homogenised in 100 mL distilled water with KCl (0.01 mol/L) for 1 min in a stomacher with filter net (Pro-media, SH-IIM; Elmex Ltd, Tokyo, Japan; stomacher bags: PX0020, Elmex, Ltd., Japan). Impurities were filtered by the filter net. These steps were conducted multiple times for all samples. The pH values were recorded using a pH meter (S210 SevenCompact™ pH meter, Mettler Toledo).

2.2.2. Instrumentally measured colour

Lightness (L*), redness (a*), and yellowness (b*) were used as indicators for surface colour of samples. The indicators were measured by a Chroma meter CR-400 (Konica Minolta Sensing Inc, Osaka, Japan), with an 8 mm diameter measuring area, a D65 illuminant and 10° standard observer, expressed in *CIE Lab* colour space (two decimal places). Five spots randomly selected for colour measurement were measured on individual samples (L*, a*, b*).

2.2.3. Total volatile base nitrogen analysis (TVB-N)

The TVB-N content in bacon was measured using a steam distillation method, in accordance with Chinese Standard GB 5009.228–2016. Samples (10 g) were ground individually by a processor (JYL-C012, Jiuyang, China). Steam distillation was carried out using a Kjeldahl distillation unit (KDN-103F, Shanghai Xian Jian., China), and TVB-N results were calculated according to the consumption of hydrochloric acid (0.01 mol/L).

2.3. Culture-dependent methods

2.3.1. Microbial enumeration and bacteria isolation

Samples (25 g) were harvested from the triplicates samples which were minced and mixed at each point, and were weighed under an aseptic environment. Then the weighed sample was homogenised in 225 mL of 1/4 Ringer's solution (Oxoid) for 2 min within a stomacher (SH-IIM, Pro·media, Japan; stomacher bags:PX0020, ELMEX, Japan) at ambient laboratory temperature. Decimal dilutions in 1/4 strength Ringer's solution (Oxoid) were prepared. Then aliquots of 0.1 mL were spread on the following growth media: Plate Count Agar (PCA, Oxoid) which was incubated at 30 °C for 48 h to achieve the total plate count and at 7 °C for 10d for the psychrophiles (separately); Violet Red Bile Glucose Agar (VRBGA, Lang Bridge) for the *Enterobacteriaceae*, incubated at 37 °C for 36 h; Man, Rogosa Sharpe (MRS) agar (Oxoid) for Lactic acid bacteria (LAB), incubated at 30 °C for 48 h; streptomycin sulphate thallous acetate cycloheximide (actidione) agar (STAA) medium (Oxoid) for *Brochothrix thermosphacta*, incubated at 25 °C for 48 h; Centrimide-Fucidin-Cepha Loridine (CFC) medium (Oxoid) to yield *pseudomonads* which incubated at 25 °C for 48 h. Rose Bengal (Lang Bridge) for yeasts and moulds incubated at 25 °C for 5 days; Baird-Parker Agar (Lang Bridge) for *staphylococci* which was incubated at 37 °C for 48 h. MRS and VRBGA plates incubated under anaerobic conditions in a 2.5 L round bottom vertical anaerobic culture bag (Hopebio). Bacterial colonies counts were expressed as the means of $\log_{10}$ CFU/g for all replicates. The lowest detection limit of the above enumeration techniques was set 2 $\log_{10}$ CFU/g. The selectivity of the growth medium was examined by performing the following rapid tests: CFC Agar was supplemented with Pseudomonas CFC Supplement

(Oxoid) and STAA agar with STAA selective supplement SR0151E (Oxoid). Baird-Parker Agar enriched with 5% potassium tellurite enrichment solution (Lang Bridge). After incubation, according to the morphological features of the colonies (appearance, size, and colours) about 10 presumptive colonies were selected from the appropriate gradient dilution plates that contained 30–100 colonies and were purified at least two times by streaking on the same medium from which the colonies were originally harvested. Cells of selected isolates were picked until pure cultures were obtained and then examined with Gram staining, microscopic observation and catalase testing. Selected isolates were homogenised into 300 µl of 50% sterile glycerol and stored for a long period at −20 °C.

2.3.2. DNA extraction and genomic sequence amplification

Genomic DNA from pure cultures was extracted by a TIANamp Bacteria DNA Kit (DP302) (Tiangen, Beijing, China) according to the manufacturer's instructions. The 16S rRNA gene fragment was amplified by polymerase chain reaction with a pair of universal primers 27F (5′- AGAGTTTGATCCTGGCTCAG -3′) and 1492R (5′- AAGGAGGTGAT CCAGCCGCA-3′) according to previous research (EDEN et al., 1991). The purified PCR products were sent to GenScript (Nanjing, Jiangsu, China) for sequencing. For strain identification, 16S rRNA gene sequences of isolates were blasted against the NCBI database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequences with more than 97% identity were considered to be the same species.

2.4. Culture-independent methods

2.4.1. DNA extraction directly from bacon samples

The bacteria cells were harvested using a previous method (Han et al., 2011) with slight modifications as follows: each sample (25 g) was homogenised in 100 mL of 1/4 Ringer's solution (Oxoid) for 2 min in a stomacher at room temperature. The homogenate was allowed to stand for 5 min, and then 40 mL of supernatant was transferred into 50 mL sterile tubes in duplicate. Each tube was centrifuged for 20 min at 800 rpm (4 °C) to precipitate and remove undesirable debris. Next, the supernatant (30 mL) was aseptically transferred into a new 50 mL sterile centrifuge tube, and re-centrifuged at 12,000 rpm for 15 min (4 °C). The two pellets were combined and transferred to a new sterile 2 mL tube and then stored at −80 °C for DNA extraction.

Bacterial genomic DNA was extracted referring to the CTAB-based method as described previously but slightly modified (Jr and Via, 1993; Via and Iii, 1995). DNA concentration was assessed using a Nano-drop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA), while 2 µl of extracts were also examined after electrophoresis on a 0.8% agarose gel.

2.4.2. Pyrosequencing for 16S rRNA

PCR amplifications of the V3-V4 16S rRNA domains were conducted by using primer pairs 341F (5′-CCTAYGGGRBGCASCAG-3′) and 802 R (5′-GGACTACNNGGGTATCTAAT-3′) containing barcodes (Polka et al., 2015). The PCR mixture (30 µL) consisted of 15 µL High-Fidelity PCR Master Mix (NewEngland Biolabs, UK), 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, 72 °C for 60 s); then a final extension at 72 °C for 5 min. The PCR products were detected with 2% agarose gel electrophoresis and purified using the Qiagen Gel Extraction Kit (Qiagen, Hilden, Germany). PCR products were mixed in equimolar ratio, then the sequencing libraries were generated using the TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, San Diego, USA) based on the manufacturer's recommendations. The Illumina HiSeq2500 platform at Novogene Bioinformatics Technology Ltd was used for 250-bp paired-end sequencing.

2.4.3. Pyrosequence data analysis

To obtain high quality clean tags, the raw tags were performed under strict filtering conditions (Bokulich et al., 2013). According to the Quantitative Insights Into Microbial Ecology (QIIME) instruction (Caporaso et al., 2010), the UCHIME Algorithm was used on the Gold database and compared with the clean tags to observe and remove chimera sequences. Thus, the effective tags were gained. The Uparse software was used to obtain the same operational taxonomic units (OTUs) of all the effective tags: similarity of the sequences greater than or equal to 97% was allocated in the same OTU (Edgar, 2013). A representative sequence for each OTU was filtered for deeper annotation. Then the taxonomy assignment and the relative abundance were produced. Data from each point were homogenised for subsequent data processing. Alpha diversity, the indices of Observed-species, Chao1, Shannon, Simpson, ACE and Goods-coverage were calculated using Qiime software (Version 1.9.1). Beta diversity, the UniFrac distances and the UPGMA clustering tree were assessed by Qiime software.

2.5. Statistical analysis

The means of meat physicochemical quality and viable counts were analysed by one-way analysis of variance (ANOVA), using SPSS 20.0 statistical software (SPSS Inc., Cary, NC, USA). The results are given as standard error (SE) of the mean. Differential analyses and results were statistically significant at $P < 0.05$. The correlations between pH, colour, TVB-N, and enumeration of microorganisms were analysed by partial least squares regression (PLSR) using the Unscrambler version 9.7 (CAMO ASA, Oslo, Norway). The pH, TVB-N, colour and viable counts were presented as the X-matrix and score values at different storage times as the Y-variable. The detailed PLSR analysis method was prepared according to the method previously described (Song et al., 2010).

3. Results

3.1. Physicochemical change

3.1.1. pH

As shown in Table 1, the initial pH of the sample was 6.46 and changed to a final pH of 5.98 after being stored for 45 days. The

Table 1

Results in pH, TVB-N and L* a* b* values of the vacuum-packaged bacon during refrigerated storage.

Physicochemical indexs	Storage time/days					
	0	7	15	22	30	45
pH	6.46 ± 0.03^{a}	6.44 ± 0.08 ^{a}	6.33 ± 0.09^{b}	6.21 ± 0.06 ^{c}	6.08 ± 0.10^{d}	5.98 ± 0.09^{e}
TVB-N (mg/100 g)	5.01 ± 0.20^{c}	3.93 ± 0.43^{d}	4.81 ± 0.19^{c}	5.78 ± 0.62^{b}	5.97 ± 0.16^{ab}	6.37 ± 0.23^{a}
L*	54.91 ± 1.64^{c}	57.04 ± 2.23^{bc}	59.49 ± 2.25^{ab}	57.97 ± 3.73^{ab}	59.51 ± 1.41^{ab}	60.88 ± 1.26^{a}
a*	16.16 ± 0.13^{a}	15.85 ± 1.03^{ab}	14.46 ± 1.34^{bc}	14.85 ± 2.01^{abc}	14.41 ± 0.58^{bc}	14.06 ± 0.81^{c}
b*	6.48 ± 0.55^{c}	7.03 ± 0.40^{abc}	7.22 ± 0.46^{abc}	7.38 ± 0.48^{ab}	7.55 ± 0.65^{a}	6.84 ± 0.58^{ab}

Note: Figures in the table are means and standard error.

$^{a, b, c, d, e}$ Means within a row refer to the significant difference ($p < 0.05$).

fluctuation of pH can be divided into two stages in Table 1: firstly, the pH value experienced a slow decrease from day 0 (6.46) to day 7 (6.44), secondly, the pH decreased rapidly from day 15 (6.33) to day 45 (5.98).

3.1.2. Bacon colour

According to Table 1, the initial lightness (L*), redness (a*), and yellowness (b*) values of the 0 day vacuum-packaged bacon were 54.91, 16.16, and 6.48 respectively. Besides, the final L*, a* and b* of bacon after 45 days' storage were 60.88, 14.06, and 6.84. Briefly, the bacon became lighter (due to increased L*) and paler (due to decreased a*). Generally, the yellowing value was increasing although having undergone significant fluctuations during storage (Table 1). Further, the conclusion can be made that the colour experienced a significant change during storage.

3.1.3. Total volatile basic nitrogen (TVB-N)

Referring to Table 1, the fluctuation of TVB-N value could be divided into two stages: in the first stage ranging from day 0 to day 7 the TVB-N value was reduced from 5.01 to 3.93, in the second stage from day 7 to day 45, TVB-N was increased from 3.93 to 6.37 (Table 1).

3.2. Microbiological analyses

3.2.1. Enumeration of microorganisms

Viable counts of the microbiological groups involved in bacon deterioration were shown at different storage points, and eight culture media under different temperature and atmosphere conditions which were used for the recovery of diverse microbial groups and/or species (Table 2). Referring the result, no visible colonies were observed (< 2 $\log_{10}$ CFU/g) on day 0 except PCA. Then, no colonies were also detected at day 7 on the VRBGA、CFC and STAA medium. Colonies on PCA and MRS medium grew more rapidly since day 15, reaching the peak on day 45. Besides, the colonies on CFC, VRBGA and BP had relatively slow growth rate, the loads there of reached 5.23, 6.08, and 6.07 $\log_{10}$ CFU/g on day 45, respectively. Colonies on STAA and Rose Bengal were counted in lower proportions throughout the experiment, which suggested that they did not play crucial roles in meat spoilage.

3.2.2. Identification of the isolates from growth media

All colonies were examined according to their morphological, biochemical (catalase test) and gram staining properties to confirm that the colonies were belong to the specific bacteria/species which the selective medium targeted. A total of 223 colonies were isolated from selective culture media, the numbers were 57 (PCA 30 °C), 56 (PCA 7 °C), 25 (MRS), 23 (BP), 22 (VRBGA), 20 (CFC) and 20 (STAA) respectively. Then all of them were identified by the 16S r RNA sequence analysis and were divided into 26 species (Table 3).

Referring to Table 3, 29 isolates of 57 (50.9%) were identified as *Leuconostoc carnosum* and *Leuconostoc mesenteroides* on PCA at 30 °C throughout the storage period. In comparison, only two isolates of 57 (3.5%) on day 45 were identified as *Lactobacillus curvatus* and *Lactobacillus sakei*. Other isolates were clustered into eight species and the most adequate species were *Rouxiella chamberiensis* and *Staphylococcus xylosus*, followed by *Carnobacterium maltaromaticum* and *Carnobacterium divergens*.

The majority of isolates were also identified as *Leuconostoc carnosum* and *Leuconostoc mesenteroides* from PCA at 7 °C, while nine species were unique when isolated. It is a remarkable fact that *Leuconostoc gelidum* was detectable only at times before day 15. Only eight isolates were identified from MRS, the dominant species were still *Leuconostoc mesenteroides* and *Leuconostoc carnosum. Weissella minor* and *Lactobacillus plantarum* were also detected, albeit in small amounts. There were five isolates isolated from BP. The top two species with highest concentration were *Staphylococcus xylosus* and *Staphylococcus vitulinus,* which were found only after day 15. *Serratia liquefaciens* and other isolates were found on VRBGA, *Rouxiella chamberiensis* and *Buttiauxella ferragutiae* as the majority of the isolates, were identified after incubation of CFC plates. *Brochothrix campestris* was the exclusive isolated microorganism from STAA. Colonies from yeasts and moulds growth medium were not isolated and identified.

3.3. Relationship between pH, TVB-N, L* a* b* and microorganisms

ANOVA-PLSR was performed to process the mean data accumulated from physicochemical index and microbiological properties. The X-matrix was designated as the measurements of pH, TVB-N, colour and viable counts of bacon at different storage times. The Y-matrix was designated as design variables based on score values at different storage times. The derived PLSR model included two significant PCs and explained 93% of the cross-validated variance. Two explained variances (50% and 100%, respectively) were indicated by the inner and outer ellipses in the plot. The resultant correlation loadings plots of PC1 and PC2 (Fig. 1) show that samples appeared to be separated along PC1, samples day 0 and day 7 on the right and samples day 15, day 22, day 30 and day 45 on the left. The variation along PC1 could be explained physicochemical and microbiological index. All of the X-matrix and two Y- matrices were placed between the inner and outer ellipses indicating that they were well explained by the PLSR model. Matrices.

3.4. Microbial communities identified by high-throughput sequencing

3.4.1. Bacterial species richness and equitability

After the V3-V4 region of the 16 rRNA was amplified, a well-defined overview of the diversity and abundance of microbial communities of

Table 2
Viable counts of different spoilage-related bacteria during refrigerated storage at 0, 7, 15, 22, 30, and 45 days.

Microbiological indexs	colony and culture medium	Storage time/days					
		0	7	15	22	30	45
Viable count ($\log_{10}$ CFU/g)	TVC (PCA 30 °C)	2.18 ± 0.18^{e}	3.51 ± 0.37^{d}	5.75 ± 1.08^{c}	7.21 ± 0.65^{b}	7.91 ± 0.61^{ab}	8.04 ± 0.61^{a}
	TVC (PCA 7 °C)	2.26 ± 0.23^{e}	3.52 ± 0.44^{d}	5.65 ± 0.92^{c}	7.09 ± 0.70^{b}	7.83 ± 0.57^{ab}	8.01 ± 0.61^{a}
	LAB (MRS)	ND	3.13 ± 0.55^{d}	5.27 ± 0.81^{c}	7.18 ± 0.43^{b}	8.05 ± 0.21^{a}	8.11 ± 0.37^{a}
	Staphylococcaceae (BP)	ND	2.20 ± 0.23^{d}	4.21 ± 0.59^{c}	5.29 ± 0.41^{b}	5.47 ± 0.45^{bc}	6.07 ± 0.42^{a}
	Enterobacteriaceae (VRBGA)	ND	ND	3.18 ± 0.67^{c}	5.34 ± 0.70^{b}	5.07 ± 0.12^{b}	6.08 ± 0.46^{a}
	Pseudomonads (CFC)	ND	ND	ND	3.90 ± 0.37^{b}	4.47 ± 0.67^{ab}	5.23 ± 0.65^{a}
	Brochothrix thermosphacta (STAA)	ND	ND	3.98 ± 0.70^{ab}	4.69 ± 0.52^{a}	4.07 ± 0.71^{ab}	3.29 ± 0.55^{b}
	Moulds and Yeasts (Rose Bengal)	ND	2.11 ± 0.12^{c}	2.50 ± 0.33^{ab}	2.18 ± 0.18^{bc}	2.64 ± 0.30^{a}	2.22 ± 0.16^{bc}

Figures in the table are means and standard error.
The letters a, b, c, d and e refer to the significant difference ($p < 0.05$).
ND: Not detected ($\log_{10}$ CFU/g < 2).
TVC: Total viable count.
LAB: Lactic acid bacteria.

Table 3
Identification of isolates recovered from the different growth media based on 16S rRNA gene sequencing analysis.

Isolates	No.	Base pairs	Closest GenBank relative	Identity (%)	Closest relative accession no.	Storage time/days					
						0	7	15	22	30	45
PCA	P1	1435	*Rouxiella chamberiensis*	98	NZ_JRWU01000013	1	0	0	1	2	2
30 °C	P2	1454	*Staphylococcus xylosus*	100	NZ_CP007208.1	1	1	0	2	2	1
	P3	1018	*Buttiauxella ferragutiae*	99	NZ_LXEQ01000067	2	1	0	0	0	0
	P4	928	*Psychrobacter sp*	98	NZ_CM003594	2	1	0	1	0	0
	P5	974	*Leucobacter chromiiresistens*	99	NZ_JH370379	2	0	0	0	0	0
	P6	1444	*Leuconostoc mesenteroides*	99	NC_008531	0	2	5	3	3	3
	P7	1422	*Corynebacterium variabile*	99	NZ_AJTR01000204	0	1	0	0	0	0
	P8	1452	*Carnobacterium divergens*	99	NZ_JQLO01000001	0	1	1	0	0	0
	P9	1450	*Carnobacterium maltaromaticum*	100	NC_019425	0	2	0	0	0	0
	P10	1440	*Leuconostoc carnosum*	99	NC_018673	0	1	4	3	3	2
	P11	1471	*Lactobacillus curvatus*	100	NZ_CP020459	0	0	0	0	0	1
	P12	1475	*Lactobacillus sakei*	100	NZ_CP022474	0	0	0	0	0	1
	Tot					8	10	10	9	10	10
PCA	P4	928	*Psychrobacter sp*	98	NZ_CM003594	2	1	0	0	0	0
7 °C	P13	1428	*Psychrobacter aquaticus*	99	NZ_AUSW01000009	4	1	0	0	0	0
	P14	1383	*Chryseobacterium jeonii*	99	NZ_JSYL01000002	2	0	0	0	0	0
	P15	1012	*Psychrobacter alimentarius*	98	NZ_CP014945	1	1	0	0	0	0
	P16	1448	*Leuconostoc gelidum*	100	NC_014319	0	1	1	0	0	0
	P17	1444	*Carnobacterium maltaromaticum*	99	NC_019425	0	0	0	1	0	0
	P10	1440	*Leuconostoc carnosum*	99	NC_018673	0	5	2	1	5	5
	P6	1444	*Leuconostoc mesenteroides*	99	NC_008531	0	0	3	5	2	2
	P8	1452	*Carnobacterium divergens*	99	NZ_JQLO01000001	0	1	1	0	0	0
	P18	1439	*Staphylococcus vitulinus*	100	NZ_AJTR01000204	0	0	0	3	0	0
	P19	1431	*Psychrobacter sp*	98	NZ_LNUO01000190	0	0	0	2	1	0
	P20	1436	*Serratia liquefaciens*	99	NC_021741	0	0	0	0	0	0
	P21	1454	*Staphylococcus xylosus*	100	NZ_CP007208.1	0	0	0	0	0	1
	P1	1435	*Rouxiella chamberiensis*	98	NZ_JRWU01000013	0	0	0	0	1	1
	Tot					8	10	8	12	9	9
MRS	P16	1448	*Leuconostoc gelidum*	100	NC_014319	0	4	0	0	0	0
30 °C	P9	1450	*Carnobacterium maltaromaticum*	100	NC_019425	0	1	0	0	0	0
	P10	1440	*Leuconostoc carnosum*	99	NC_018673	0	0	3	4	1	1
	M1	1480	*Weissella minor*	99	NZ_JQCD01000006	0	0	1	0	1	0
	M2	1463	*Lactobacillus plantarum*	99	NC_004567	0	0	1	1	1	0
	P6	1444	*Leuconostoc mesenteroides*	99	NC_008531	0	0	0	0	2	2
	P11	1471	*Lactobacillus curvatus*	100	NZ_CP020459	0	0	0	0	0	1
	P12	1475	*Lactobacillus sakei*	100	NZ_CP022474	0	0	0	0	0	1
	Tot					0	5	5	5	5	5
BP	P9	1450	*Carnobacterium maltaromaticum*	100	NC_019425	0	0	3	1	1	0
37 °C	P8	1452	*Carnobacterium divergens*	99	NZ_JQLO01000001	0	0	2	0	0	1
	P2	1454	*Staphylococcus xylosus*	100	NZ_CP007208.1	0	0	0	3	3	4
	P18	1439	*Staphylococcus vitulinus*	100	NZ_AJTR01000204	0	0	0	1	1	2
	Tot					0	0	5	5	5	8
VRBGA	P4	928	*Psychrobacter sp*	98	NZ_CM003594	0	2	1	0	0	0
37 °C	V1	1444	*Enterobacter sp*	98	NC_009436	0	0	4	1	0	1
	P20	1436	*Serratia liquefaciens*	99	NC_021741	0	0	0	3	1	3
	P8	1452	*Carnobacterium divergens*	99	NZ_JQLO01000001	0	0	0	1	0	0
	P3	1012	*Buttiauxella ferragutiae*	99	NZ_LXEQ01000067	0	0	0	0	4	1
	Tot					0	2	5	5	5	5
CFC	P20	1446	*Serratia liquefaciens*	99	NC_021741	0	0	0	1	0	0
25 °C	C1	1441	*Obesumbacterium proteus*	99	NZ_CP014608	0	0	0	0	1	0
	P1	1443	*Rouxiella chamberiensis*	99	NZ_JRWU01000013	0	0	2	2	2	2
	P3	1017	*Buttiauxella ferragutiae*	99	NZ_LXEQ01000067	0	0	3	2	2	3
	Tot					0	0	5	5	5	5
STAA	S1	1452	*Brochothrix campestris*	99	NZ_AODH01000087	0	0	5	5	5	5
25 °C	**Tot**					0	0	5	5	5	5

smoked bacon during storage was represented (Table 4). A total of 8548 operational taxonomic units (OTUs) were finally achieved by several steps. Firstly a total of 2,612,194 sequences (raw tags) from 30 samples were sequenced using Illumina HiSeq. Secondly, quality filtering was conducted and the chloroplast sequence was removed. As a result, 2,099,851 high quality sequences (clean tags) remained (Table 4). Thirdly, a chimeral filtration and quality control were carried out thus obtaining a total of 1,998,591 sequences (effective tags). Finally, a classification step was conducted and gained OTUs as Table 4 represented. Table 4 also indicates that each sample had a number of OTUs ranging from 148 to 474 per sample. OTUs, Shannon, Simpson, and Chao1 showed significant differences among their own groups. The results of ANOVA test (5% level) were OTUs ($F = 12.714$, $p = 0.000$), Shannon ($F = 11.391$, $p = 0.000$), Simpson ($F = 6.212$, $p = 0.000$), Chao1 ($F = 11.784$, $p = 0.000$), and ACE ($F = 13.076$, $p = 0.000$). Additionally, all five indices showed a similar trend of decline.

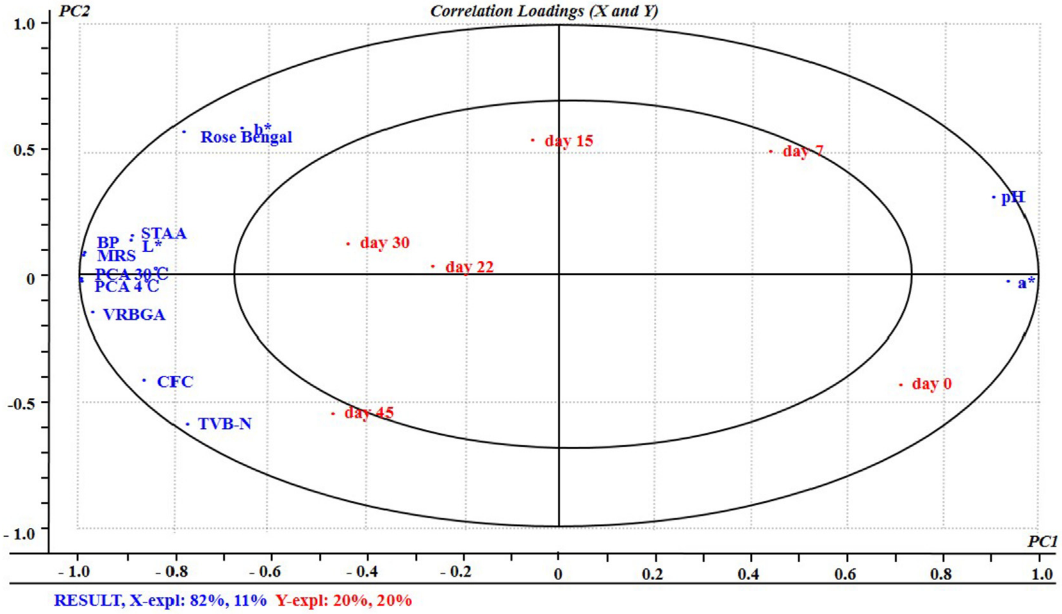

Fig. 1. ANOVA–PLSR correlation loadings plot of pH, TVB-N, L* a* b* and viable counts for the different stages. The model was derived from pH, TVB-N, colour and viable counts as the X-matrix and score values at different storage time as the Y-matrix. Ellipses represent $r^2 = 0.5$ and 1, respectively.

Table 4
Alpha diversity estimation of the 16S rRNA gene libraries by sequencing on an Illumina HiSeq2500 platform in vacuum-packaged smoked bacon.

Sample Group	Sample name	Total Tags	OUTs	Average length	Shannon	Simpson	Chao1	ACE	Goods coverage
day 0	day 0.1	68,628	269	422	3.727	0.823	334.022	349.378	0.997
	day 0.2	61,319	344	414	5.006	0.914	410.725	421.400	0.996
	day 0.3	64,262	412	416	5.226	0.931	521.018	520.978	0.995
	day 0.4	70,829	474	412	5.870	0.958	527.882	536.741	0.996
	day 0.5	70,406	418	412	5.889	0.958	459.714	469.906	0.997
day 7	day 7.1	62,990	452	412	5.725	0.940	523.127	538.093	0.996
	day 7.2	72,515	358	412	4.839	0.879	400.387	412.784	0.997
	day 7.3	73,362	429	411	5.411	0.937	446.353	464.132	0.997
	day 7.4	76,600	296	424	3.728	0.738	363.517	396.354	0.996
	day 7.5	78,308	417	412	5.655	0.954	452.500	465.504	0.997
day 15	day 15.1	65,017	326	414	4.491	0.852	359.768	368.198	0.997
	day 15.2	67,803	205	426	2.209	0.560	288.824	314.043	0.997
	day 15.3	67,063	354	414	4.740	0.878	384.500	397.969	0.997
	day 15.4	63,028	259	427	2.145	0.546	346.073	351.384	0.996
	day 15.5	76,317	366	415	4.651	0.884	457.062	450.929	0.996
day 22	day 22.1	68,060	262	427	2.805	0.670	320.132	352.956	0.997
	day 22.2	65,287	261	417	2.919	0.647	296.594	324.476	0.997
	day 22.2	66,739	211	426	2.789	0.759	253.977	271.856	0.997
	day 22.4	66,879	213	427	2.632	0.666	284.000	300.073	0.997
	day 22.5	63,296	236	427	2.725	0.678	299.068	315.356	0.997
day 30	day 30.1	69,934	194	427	2.544	0.672	245.107	239.250	0.998
	day 30.2	60,184	165	427	2.429	0.632	188.786	197.622	0.998
	day 30.3	57,350	250	424	3.077	0.712	331.618	334.804	0.997
	day 30.4	42,124	272	426	3.268	0.794	338.733	346.176	0.997
	day 30.5	64,245	200	424	3.078	0.787	273.966	278.041	0.997
day 45	day 45.1	67,888	207	426	2.814	0.671	258.656	271.582	0.997
	day 45.2	68,149	148	428	2.712	0.729	203.038	225.010	0.998
	day 45.3	61,962	166	428	2.244	0.590	217.107	229.935	0.998
	day 45.4	65,484	162	428	2.254	0.581	228.957	236.393	0.998
	day 45.5	72,563	222	426	2.977	0.763	295.026	314.193	0.997

Alpha diversity indices, such as observed species, Shannon and chao1, were represented in the form of box plots (Fig. 2a, b, and 2c, respectively). According to Fig. 2, the three indices were significantly higher ($P < 0.05$) on day 0 and day 7 compared with other storage and no significant differences between them, indicated that bacteria were most community diversity at initial storage. Subsequently, the value of three indices significantly decreased ($P < 0.05$) continuously with increasing storage time, and reached the lowest bacterial diversity on day 45.

3.4.2. Microbial community composition

The diversity of bacteria during storage was analysed by 16S rRNA and described as relative abundance at phylum and genus level in Fig. 3. According to Fig. 3a, three phyla were the majority component of the microbiota. The Firmicutes were steadily increased during the whole storage period from 29.90% on day 0–82.12% on day 45. Proteobacteria showed the tendency of continuous decrease during storage. Proteobacteria was the dominant bacteria on day 0 which had a relative abundance of 42.82%. Then a slight increase was observed on

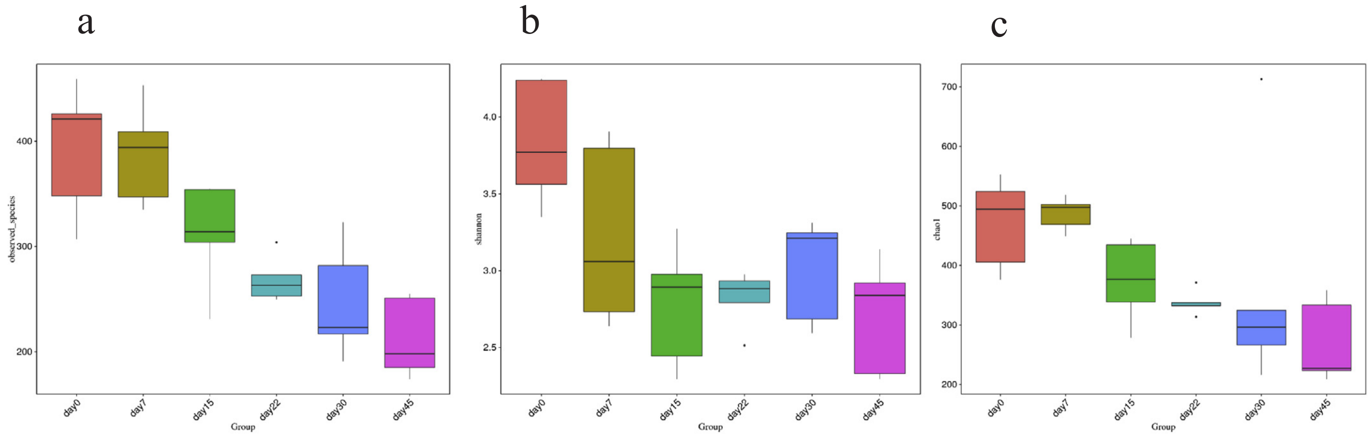

Fig. 2. Box plot of index difference between groups of observed species (a), Shannon (b) and Chao1 (c) at different storage time points. Wilcox rank sum test and Tukey test were used to analysis the differences and significant in species diversity between groups.

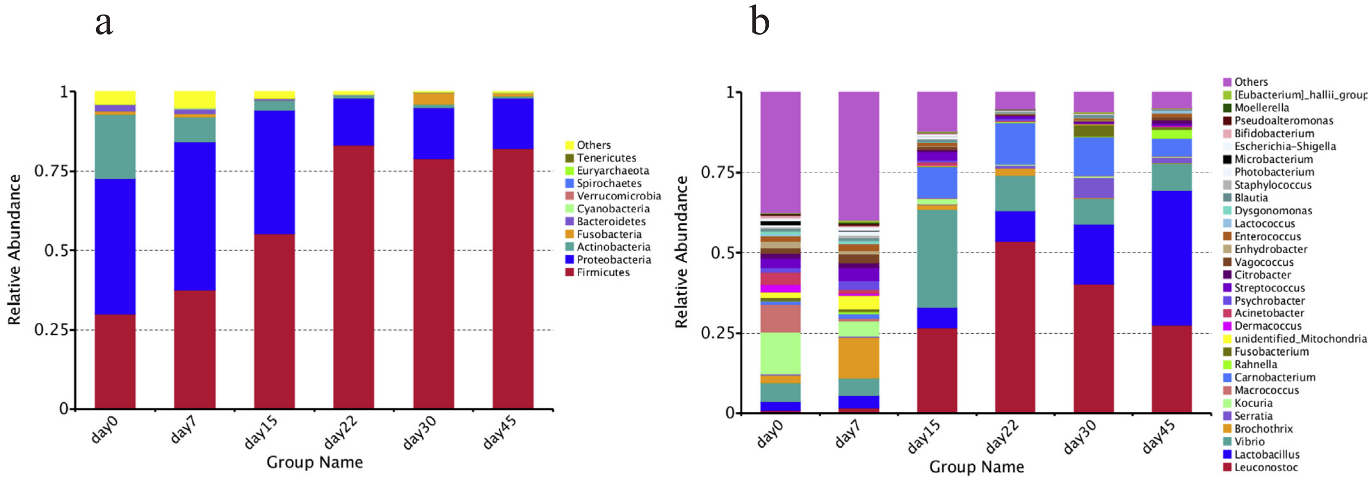

Fig. 3. Bacterial community structure variation in different stages. The relative abundance species of bacteria at the (a) phylum and (b) genus level is shown. Lower the top 10 abundance of the phyla and the top 30 abundance of the genus was merged into others. Each bar represents the relative abundance of each sample. Each colour represents a particular phylum or genus. The numbers associated with the sample names indicate the sampling time.

day 7 to a relative abundance of 46.57%. Nevertheless, a striking similarity was observed since day 7 as the relative abundance decreased to 15.82% on day 45. Actinobacteria mainly existed before day 15 and the highest proportion was 20.26% at day 0. Afterwards, it decreased continuously to reach 7.93% at day 7, 3.03% at day 15, and almost disappeared thereafter. Other phyla were less dominant which include Fusobacteria (0.09%–3.58%), Bacteroidetes (0.18%–2.01%), Cyanobacteria (0.00%–0.28%), and the rest had a relative abundant of less than 0.05%.

At the genus level, 336 different bacterial genera were detected in total, among which an overview of the relevant top 30 was represented (Fig. 3b). Referring to Fig. 3b, the composition of microbiota underwent significant fluctuations. Although bacterial counts was small at the initial storage stage at day 0 and day 7 as shown above, the diversity of microbes, and their abundance, was considerable. During this stage, the proportion of each species was not advantageous. At day 0, the predominant populations were *Kocuria* (13.17%), *Macrococcus* (8.57%), *Vibrio* (5.90%), and *Brochothrix* (2.43%); however, it was noteworthy that *Lactobacillus* (2.60%) and *Leuconostoc* (0.90%) were all at low abundance. The composition of the microbiota kept changing. On day 7 the dominant species were *Brochothrix* (12.77%), *Vibrio* (5.42%), *Kocuria* (4.47%), *Lactobacillus* (3.74%), and *Leuconostoc* (1.74%). Further changes continued, *Vibrio* and *Leuconostoc* were the most predominant on day 15, which accounted for 30.67% and 26.70% respectively (followed by *Carnobacterium* (9.65%) and *Lactobacillus* (6.24%)). *Leuconostoc* increased significantly to 53.72% on day 22 which held an absolute advantage. *Carnobacterium* (12.94%) and *Lactobacillus* (9.44%) were slightly increased, *Vibrio* (10.96%) with others were all decreased. Since day 30, *Leuconostoc* and *Lactobacillus* accounted for the majority among all genera, reaching 69.41% on day 45. Interestingly, *Leuconostoc* decreased rapidly from the highest abundance (on day 22) to 35.2% (day 30) and 27.48% (day 45), respectively. *Lactobacillus* rapidly increased from 15.2% on day 30–45.1% on day 45. On day 45, the predominant species were *Lactobacillus* (41.93%), *Leuconostoc* (27.48%), *Vibrio* (8.43%), and *Carnobacterium* (5.52%); however, it should be pointed out that *Serratia* (6.28%) and *Fusobacterium* (3.58%) had an occasional increase at day 30 after when then become negligible on day 45. *Rahnella* and *Lactococcus* showed an unexpected increase on day 45.

Firmicutes, Proteobacteria, and Actinobacteria comprise the most of the top 100 genus ranking in terms of abundance (Fig. 4). Notably, *Leuconostoc* and *Lactobacillus* were the most predominant genus and have the closest genetic relationship during the whole storage period. *Carnobacterium* and *Brochothrix* were closer in phylogenetic tree indicating a near-taxonomical relationship.

3.4.3. Microbial diversity changes with storage time

A flower plot is constructed to analyze the similarities and differences among the microbiota (Fig. 5). According to Fig. 5 and 231 groups, which were most common to all groups, were classified as core

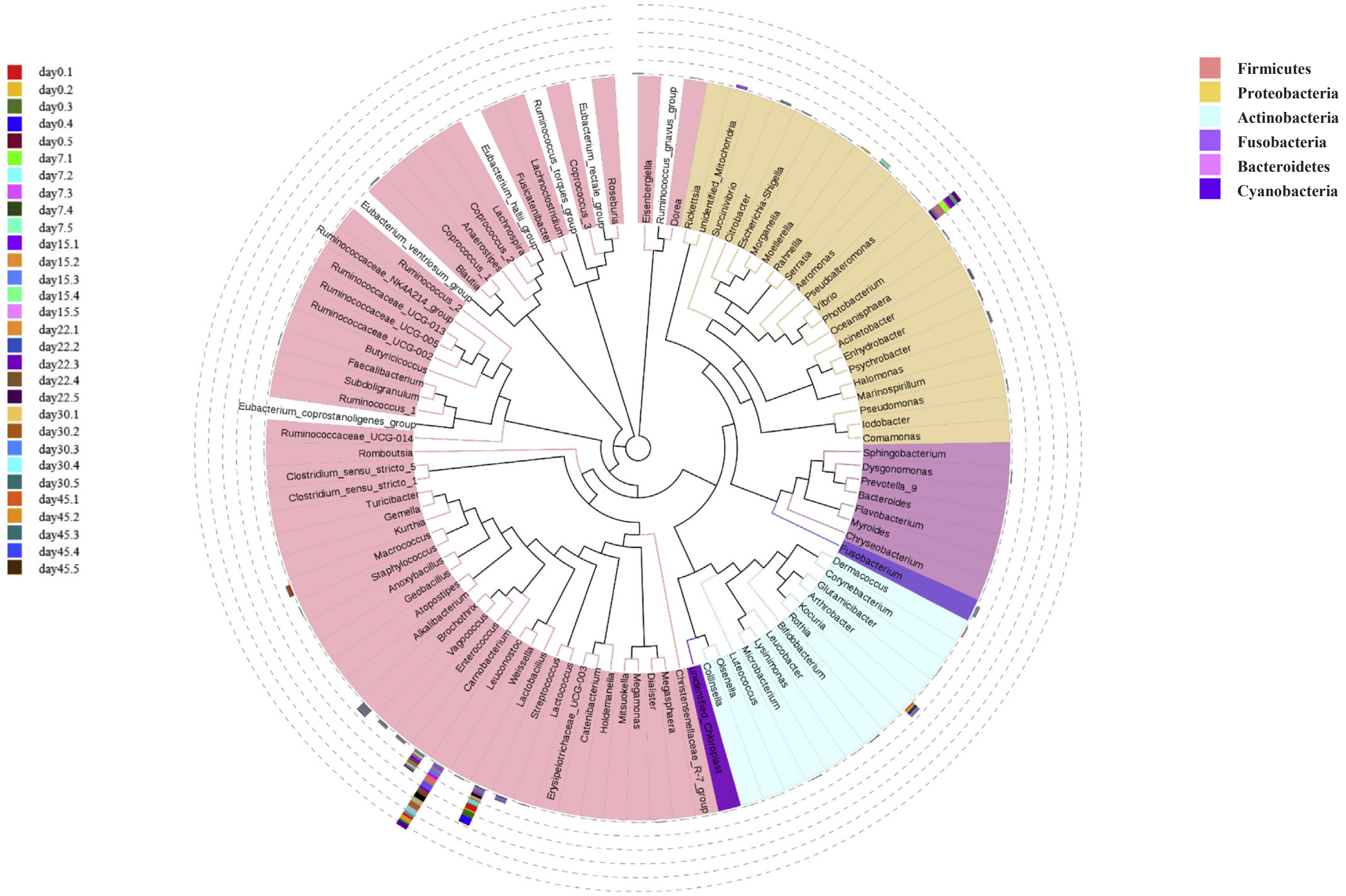

Fig. 4. Phylogenetic tree of representative sequences of species belonging to the top 100 genera. Each colour represents a particular phylum. The colour of the branch represents the corresponding phylum.

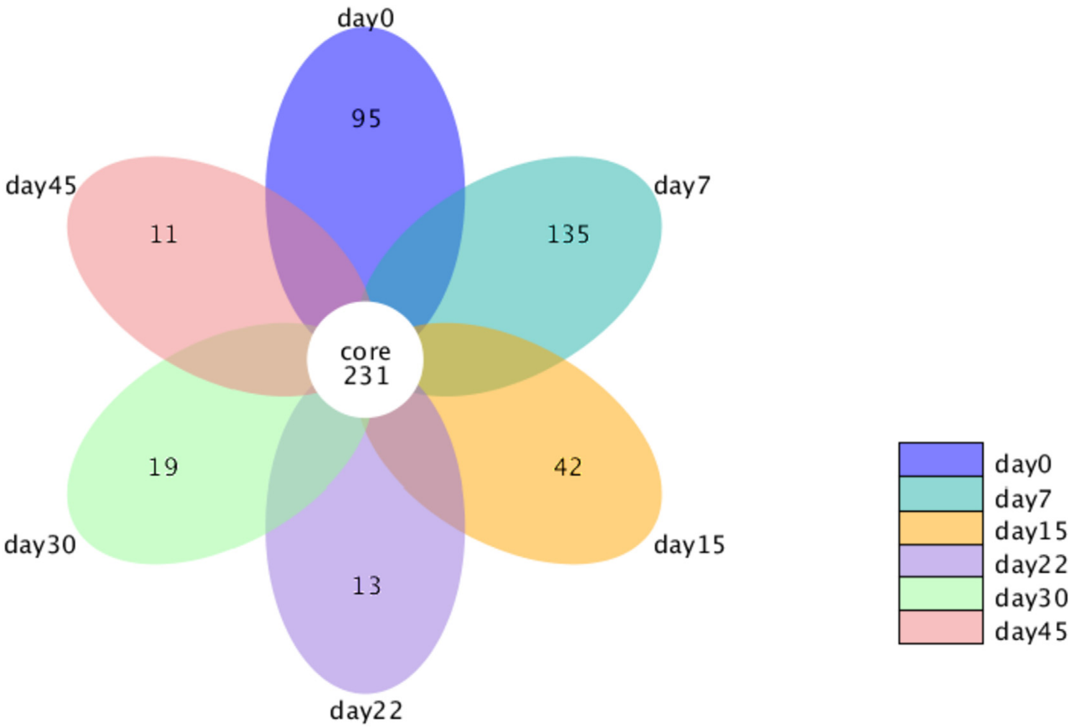

Fig. 5. Flower figure showing the unique and shared OTUs of the bacterial communities across different storage time. Only more than 5 groups the flower figure can be shown. The figure drawingwas carried out after homogenization of all the sample.

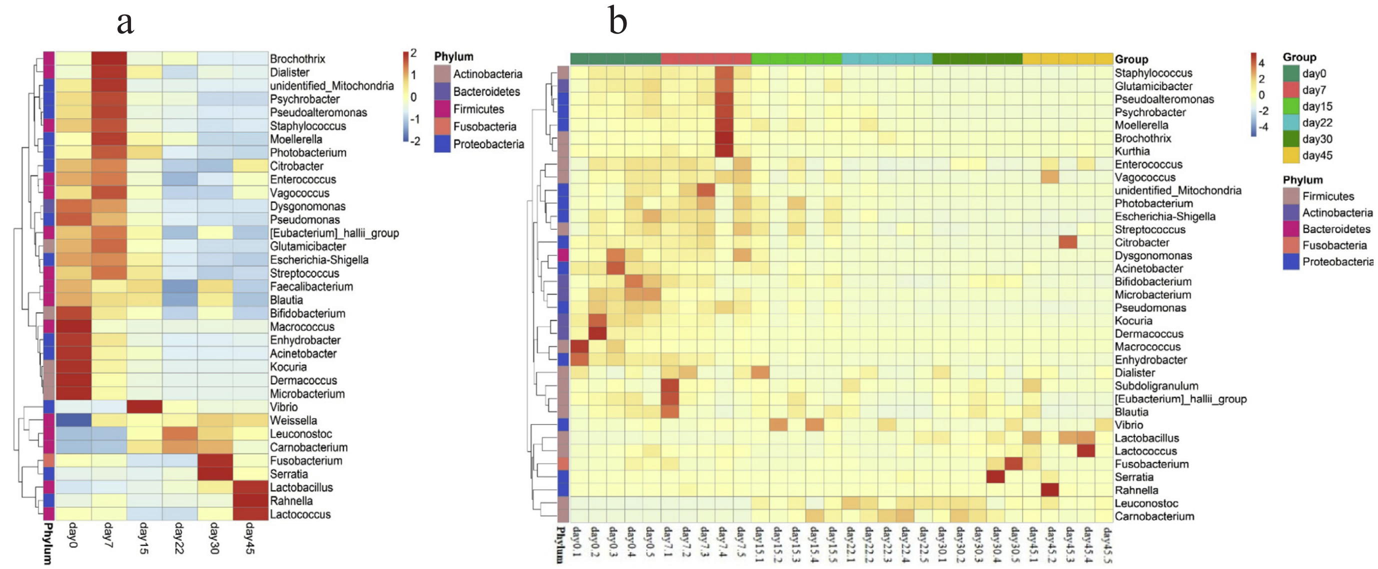

Fig. 6. Heatmap showing the changes in the microbial communities of smoked bacon packaged in a vacuum-packaged during storage. Select the bacteria whose relative abundances were top 35 at the genus level obtained in this study were specifically indicated, (a) group (b) no group. The heatmap is colour-coded based on row Z-scores. The groups heatmap were homogenization of numerical values of Corresponding day.

OTUs. The core OTUs accounted for 20.44% of the total sequence. Besides core OTUs, there were 95 unique OTUs on day 0. The number increased to 135 on day 7 which was the peak value. Afterwards, although there was a slight increase on day 30 the unique value generally decreased to its minimal value of 11 on day 45.

A heat map of the top 35 genus-level phylotypes was constructed which showed differences in bacterial composition and dynamics among the six storage times (Fig. 6): a deeper colour represented higher relative abundances. Specifically, the relative abundance of various genera (e.g. *Kocuria*) on day 0 and day 7 (e.g. *Brochothrix*) was significantly higher than that at other storage times (Fig. 6a). The most abundant observed genera were *Vibrio*, *Weissella, Leuconostoc, and Carnobacterium* at intermediate storage times. Simultaneously, the proportion of organisms including *Serratia* and *Fusobacterium* showed a sudden increase on day 30. *Lactobacillus, Rahnella,* and *Lactococcus* had the highest abundance on day 45 (Fig. 6a). Tracing the individual storage time, *Serratia, Rahnella, Fusobacterium,* and *Lactococcus* were individually increased but not across all storage times (Fig. 6b). Compared to the *Leuconostoc, Lactobacillus*, and *Carnobacterium* microbial communities, these were changed at all storage times.

4. Discussion

In this study, the changes in bacon quality correlated indicators pH, TVB-N, colour, microbial enumeration, and microbial communities of bacon were assessed, which provided a relationship between the microbiological and physicochemical index. According to Fig. 1, pH and a* are located in the positive region along PC1 and show a negative correlation with storage time, while the viable counts, TVB-N and L*, b* are located in the negative regions of PC1 and show a positive correlation with storage time. This phenomenon might have been caused by the growth and reproduction of microorganisms like LAB, *Brochothrix thermosphacta* and others were connected with the production of sour, discoloration, greening and slimes, which negatively affect the colour and pH of vacuum packed meat product (Comi et al., 2016; Comi and Iacumin, 2012; Samelis et al., 2000a). LAB played a major role in the production of organic acids - mainly lactic acid (Ammor and Mayo, 2007), which could result in a rapid and large pH decrease and sour spoilage with the prolongation of storage time. TVB-N has been used as a spoilage indicator in several meat and meat product, which is the main product of protein decomposed by spoilage bacteria (Huang et al., 2014; Tian et al., 2017). In this study, TVB-N values are positive correlation with the increase in the number of spoilage microorganisms during storage. The pH and a* values maintained stable and showed commercial quality was preferable on day 0 and day 7 then they fell rapidly during the refrigeration period. It was obvious that day 45 was co-varied with microorganisms and TVB-N, which indicated that these variables were positively correlated and of relevance. To conclude, the quality of bacon showed a declining tendency with prolonged storage.

In smoked bacon processing, the liquid smoke comes from sawdust and spices were added, which contain chloroplasts. Chloroplasts are evolutionarily descended from bacteria, the 16S genes share high sequence similarity sequences between the two (Hanshew et al., 2013). Plant chloroplast 16S rRNA have high homology to bacterial 16S rRNAs (Sun et al., 2008). The same regions used for primer design allows broad bacteria and chloroplast 16S amplification (Hanshew et al., 2013). In this study, the universal 16S primers targeting hypervariable regions V3-V4 were used for high-throughput sequencing. Chloroplast 16S genes have two base pair mismatches at positions 798 and 799 (Chelius and Triplett, 2001), which exert a major influence on data sets analysis. Mismatches between them are commonly amplification errors induced by contamination thus being a major drawback for the culture-independent community analysis of bacterial (Rastogi et al., 2010). Chaillou et al. (2015) removed 58% of all OTUs associated with the use of spices in poultry sausage samples which were caused by chloroplast contamination, then an accurate bacterial communities was achieved. Sequences of chloroplast origin were also confirmed and removed from the data set (Bengtsson et al., 2012; Mason et al., 2014). In this study, the DNA extraction was a mixture of both plant and bacterial types thereof which contained chloroplasts causing contamination, thus these OTUs were also removed from the result to generate an accurate α-diversity.

Although the initial bacteria loads were negligible with no dominant species from day 0 to day 7, greater diversity was observed. Approximately 18 species were isolated, such as *Psychrobacter* spp, *Buttiauxella ferragutiae*, but none of them was a majority species in the population (Table 3). High-throughput sequencing (HTS) showed the same trend at the initial stages (Fig. 3). All indicators of microbial Alpha Diversity (Shannon, Simpson, Chao1, and ACE) showed that the microbiota diversity were high during the first 7 days of storage and declined rapidly with prolonged storage time (Table 4).

At order level, *Enterobacteriales, Bacillales, Lactobacillales,* *Micrococcaceae, Pseudomonadales* and *Vibrionales* were the top six species (more than 5% individually) before day 7. This is in accordance with the literature which indicated that these species are major spoilage microorganisms originated from raw meat (Doulgeraki et al., 2012). Presumably due to the mild heat treatment during processing at below 72 °C. Under mild heat treatment microorganisms derived from raw meat and excipients cannot be completely killed and post-heat treatment recontamination later; however, over longer storage time, most of the initial microorganism loads descend and only *Lactobacillales* and *Vibrionales* increased at day 15. *Lactobacillales*, *Vibrionales* and *Enterobacteriales* were top three species (more than 5% individually) on day 45.

Due to an anaerobic environment created by vacuum packaging, *Lactobacillales* and *Vibrionales* are facultative anaerobes which are able to grow. The relative abundance of *Vibrio* increased rapidly to 31.41% and it became the predominant microbiota on day 15, after that it fell rapidly and almost disappeared at the end of storage except for one time for an individual batch on day 45.5 (Fig. 6). *Vibrio* could grow and dominated spoilage microorganisms during storage of meat/meat products (Nychas et al., 2007; Xiao et al., 2013). This may be explained thus: firstly, LAB's population is positively related to storage time and *Vibrio* has to compete with dominant LAB during late storage thus causing the aforementioned (Khouadja et al., 2017; Koga et al., 1998). Secondly, the unfavourable growth conditions induced by pH value and NaCl changes lead to the demise of *Vibrio* (Xiao et al., 2013). *Vibrio* has been detected in cured meats and related to spoilage with meat packed under vacuum or modified conditions (Nychas et al., 2008), and it may also contribute to spoilage of bacon; however, *Vibrio* was not isolated successfully on multiple media, this may be due to the strict conditions *Vibrio* required for growth.

Previous studies have reported that LAB were the main spoilage microbial species on a variety of vacuum-packaged cooked meat and other processed meat (Borch et al., 1996; Chenoll et al., 2007; Hamasaki et al., 2003; Korkeala and Björkroth, 1997; Samelis et al., 2000a). In the present work, LAB dominated the microbiota after 7 days' storage (Fig. 3b). The rapid growth of LAB may be due its facultative anaerobic characteristic, and the higher competitivity compared to other bacteria under vacuum packaging. LAB are Gram-positive bacteria and mainly consist of *Lactobacillus, Lactococcus, Leuconostoc, Enterococcus,* and *Pediococcus* (Holzapfel et al., 2001). Based on the results of HTS, *Lactobacillus* and *Leuconostoc* were not significantly higher than other microorganisms at the initial stage (3.5% on day 0, 5.5% on day 7, respectively) then dominated the microbiota at different stages. *Leuconostoc* became the dominant bacteria from metaphase to terminal. Later, the *Lactobacillus* became the overwhelming genus at the end of the storage period (Fig. 3b). A strain or a microbial group collectively was all responsible for food spoilage: however, not all species or strains are responsible for spoilage. Spoilage is generally attributed to the dominant microbiota, usually referred to as specific spoilage organisms (SSO) (Casaburi et al., 2015). The LAB of vacuum-packed cured, cooked meat products, *Lactobacillus sakei, Lactobacillus curvatus, Leuconostoc gelidium, Leuconostoc carnosum*, *Leuconostoc mesenteroides and Weissella viridescens* were frequently found to be the most predominant reference species (Borch et al., 1996; Samelis et al., 2000a), they could probably constitute the SSOs (Pothakos et al., 2014). Hu et al. (2009) found that *Lactobacillus sakei, Lactobacillus curvatus, and Leuconostoc mesenteroides* were the dominant LAB microbiota during vacuum-packed cooked ham storage. *Leuconostoc carnosum* was identified as the SSOs in vacuum-packaged, sliced, cooked ham (Björkroth et al., 1998). Yi et al. (2017) have found that *Leuconostoc* and others might have correlation with spoilage of bacon using metagenomic analysis. Another recent study also reported that *Leuconostoc mesenteroides* was the predominant species, and responsible for the spoilage of cooked bacon under vacuum package (Comi et al., 2016). In the current study, the result showed that the major components of LAB narrowed down to certain species including *Leuconostoc (*mainly *Leuconostoc mesenteroides* and *Leuconostoc carnosum)* and *Lactobacillus* (mainly *Lactobacillus plantarum*, *Lactobacillus curvatus* and *Lactobacillus sakei*) by culture-dependent and culture-independent methods, which were recognized as SSOs may be associated with the spoilage of smoked bacon during storage; however, the deterioration mechanisms and characteristics thereof need further investigation.

In addition, HTS showed that *Lactobacillus* steadily increased after day 7. Significant changes occurred alternately in the predominant microbiota of *Lactobacillus and Leuconostoc* on day 45, *Lactobacillus* underwent a dramatic increase and became the dominant bacteria (Fig. 3b); however, *Lactobacillus curvatus* and *Lactobacillus sakei* were only found on day 45 by traditional pure culturation and identification, *Lactobacillus plantarum* was isolated from day 15 to day 30 (Table 3). *Leuconostoc mesenteroides* and *Leuconostoc carnosum* are major species under *Lactobacillus* while *Lactobacillus plantarum*, *Lactobacillus curvatus* and *Lactobacillus sakei* accounted for relatively small percentages. Possible reasons for this are as following: *Lactobacillus curvatus* and *Lactobacillus sakei* were almost nonexistent before day 45; the limit of the pure cultivation. Further investigations to identify the species of *Lactobacillus* need to be carried out.

Furthermore, *Carnobacterium* may have contributed to spoilage by way of their proliferation or metabolism. *Carnobacterium* (including the species *Carnobacterium maltaromaticum* and *Carnobacterium divergens*) could be isolated from multiple media due to its specific adaptability (Table 3). The abundance of *Carnobacterium* only reached 1.00% before day 7 and significantly increased to 12.94% on day 22 (Fig. 3b). Then, *Carnobacterium* reached its stationary phase which declined but remained at a relatively high level. Previous studies have shown that *Carnobacterium divergens* and *Carnobacterium maltaromaticum* were frequently isolated from a variety of foods, such as meat, fish and dairy products, due to their psycrophilic anaerobic characteristics, and they are frequently predominant members of the microbial community of raw meat (Casaburi et al., 2011; Leisner et al., 2007). Previous research also found that *Carnobacterium divergens* and *Carnobacterium maltaromaticum* also have been detected in a variety of processed meat products, including cured pork bacon, ham, cooked poultry, fermented sausages and other meat products (Leisner et al., 2007). Although the organisms are frequently isolated from these processed meat products, they are rarely present in large numbers, the terminal spoilage of cured, cooked meats is mainly caused by LAB (Chenoll et al., 2007; Samelis and Blackburn, 2006). The result in this article confirms this (Fig. 3). *Carnobacterium divergens* and *Carnobacterium maltaromaticum* are considered to possess extensive potential for meat spoilage and are important players in bacon deterioration dynamics.

Besides LAB, meat spoilage is often associated with Gram negative bacteria (e.g. *pseudomonads*, *Enterobacteriaceae* and *Shewanella putrefaciens*) and some Gram positive bacteria (e.g. *clostridia* and *Brochothrix thermosphacta*) (Casaburi et al., 2015; Nychas et al., 2007). In the present study, *Brochothrix* (mainly *Brochothrix campestris*) was low in abundance throughout the period of storage, although its abundance showed a large increase on day 7 then dropped; however, *Brochothrix* was not the important member of the spoilage association microbial community, this was in agreement with the findings reported in the literature for vacuum-packaged cooked ham (Samelis et al., 2000a). *Brochothrix* may be inhibited by the accumulation of hydrogen peroxide that is produced by LAB (Cayré et al., 2005).

The abundance of *Serratia* was insignificant throughout the storage period, however, it experienced an increase on day 30, traced back to the storage points only at one point of an individual batch (day 30.4) of *Serratia* which underwent dramatic change (Fig. 6b). The abnormal samples were than isolated and identified in which *Serratia liquefaciens* was detected (Table 3). Similarly, *Brochothrix* on day 7.5, *Fusobacterium* on day 30.5, *Rahnella* on day 45.2 and *Lactococcus* on day 45.4 all experienced a similar situation; however, due to the limitations of time and labour no further research was carried out, thus limiting the experimental work reported here. Due to the low concentration, and the rarity of occurrence, they may not be the main contributors to the spoilage of bacon. *Lactococcus* has been isolated from various types of cooked meat products and identified as a frequent source of spoilage (Barakat et al., 2000; Hamasaki et al., 2003). *Serratia* and *Rahnella* are typical psychrotrophic *Enterobacteriaceae* recovered from vacuum-packed meat, and frequent members of the spoilage microbiota (Gram et al., 1999); however, levels of more than 6 $\log_{10}$ CFU/g are believed to be sufficient to cause quality defects. *Fusobacteria* was another microorganism can be discovered in red meat and ham which causes food safety concerns (Ge et al., 2017; Zhu et al., 2015). Overall, they all could be considered as potential contributors to the spoilage of bacon, but again, the low concentration suggests that they were not the main organisms related to spoilage during this study.

Increasingly health-conscious consumers demand for high quality foods, such as improved food safety, nutritious, fresh appearance, natural and tasty. To combine these demands without compromising safety, various new preservation technologies such as non-thermal inactivation technologies (mainly high hydrostatic pressure (HHP)), natural antimicrobial compounds and bio-preservation of cooked meat products have been investigated for controlling the spoilage organisms and extending the shelf life (Comi et al., 2016; Devlieghere et al., 2004; Huang et al., 2017; Metaxopoulos et al., 2002). HHP is an efficient method for delaying the growth of spoilage microorganisms in the sliced vacuum-packed meat products (Garriga et al., 2004). The antimicrobial activity of spice extracts against pathogenic and spoilage bacteria in vacuum packaged slices ham (Zhang et al., 2009). *Lactococcus lactis* and *Lactobacillus sakei* as bio-protective culture were able to reduce the risk of *Leuconostoc mesenteroides* spoilage of cooked bacon (Comi et al., 2016). Where-after combined strategies were considered to inhibit the growth of spoilage organisms in smoked bacon.

Meat spoilage is a complex phenomenon contacted with microorganisms activity, single bacteria species and interactions among various bacterial species. The above observations are essential in comprehending the dynamics of the microbial community of smoked bacon storage; however, further studies are needed to monitor the specific characteristics of the spoilage-associated species isolated from bacon, and inhibit bacterial growth for extending the shelf life.

5. Conclusions

Both culture-dependent (microbial cultivation and 16S rRNA gene sequencing) and culture-independent methods were applied to explore the microbial community of vacuum-packed bacon. A total of 26 isolates were identified from selective growth media by traditional pure cultures. High-throughput sequencing analysis indicated that a total of 336 different genera were obtained from all samples. The results indicated that the microbiota diversity achieved its maximum at the early storage period and decreased with increased storage time. The use of the culture-independent method highlighted the occurrence of species that were not detected by plating. Many microorganisms were abundant but diminished in number at the middle stage and were difficult to detect at the end of the storage period. *Leuconostoc* (mainly *Leuconostoc mesenteroides,* and *Leuconostoc carnosum)* and *Lactobacillus* (mainly *Lactobacillus plantarum*, *Lactobacillus curvatus,* and *Lactobacillus sakei*) commonly dominated the middle-late stages after day 15. *Leuconostoc* became the dominant bacteria at the storage metaphase and in the later stage of the storage period, while *Lactobacillus* bacame an overwhelming dominant genus at the end of the storage. They were recognized as the SSOs and may be related to the spoilage of bacon. *Brochothrix, Serratia, Fusobacterium, Rahnella* and *Lactococcus* were occasional components at some storage points of individual batches and may also be associated with spoilage. Further investigations are needed to confirm the nature of the SSOs isolated from the product to reveal the character occurrence of deterioration, and develop appropriate technologies for prolonging the shelf life.

Conflicts of interest

The authors have no conflict of interest.

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