Correlation between the bacterial community and flavour of fermented fish

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Abstract

Common carp is a fish species of economic importance in China; traditionally it is mostly salt-fermented. In the fermenting process, the bacterial community of spontaneously fermented fish is important for its flavour and quality. However, very few studies have been conducted about the relationship between bacterial community and development of flavour involved in the salt-fermentation of carp. Therefore, we explored this relationship by determining the flavour components, including amino acids, and changes in volatile flavour and bacterial metabolite. Samples were taken during fermentation on the days 0, 10, 20, 30, 40 and 50. The second-generation 16S recombinant DNA (rDNA) sequencing was performed to analyze the composition of bacteria. Contents of amino acids were determined by reverse-phase high-performance liquid chromatography combined with ultraviolet detection. The volatile components were analyzed with solid-phase microextraction–gas chromatography–mass spectrometry. *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, and *Staphylococcus* were the dominant bacteria. The bidirectional orthogonal partial least squares approach was used to analyze the correlation between bacterial succession and flavour component dynamics. This study would help to better understand the role of bacteria in the fermented fish meat flavour and support the industrial production of fermented fish.

Keywords: fermented fish; bacterial community; flavour

Introduction

Fresh fish is one of the most highly perishable foods; its quality deteriorates rapidly during storage and handling, which severely limits the shelf-life of the product (Sallam, 2007). For this reason, there are many traditional methods of fish preservation, including salt-fermentation (Jung *et al.*, 2013). Fermented fish is generally prepared by spontaneous (approximately) 1-month fermentation of salted whole or sectioned fish (Ji *et al.*, 2017). Fermented fish contains proteins, carbohydrates, free amino acids, and a relatively low content of fat and biogenic amines (Wang *et al.*, 2014). During the fermentation process,

the fermented fish gains favourable flavour and extended shelf-life due to the co-functioning of complex bacterial communities (Dai *et al.*, 2013). The salt-fermentation of fish is one of the oldest traditional preservation methods and it has remained in use in many parts of the world. Similar low-salt fermented fish products are widely produced and consumed in Japan, Southeast Asia, and Africa (Kouakou *et al.*, 2012; Marui *et al.*, 2014; Paludan-Müller *et al.*, 2002; Takahashi and Kimura, 2010).

Traditional solid-state fermented fish is naturally fermented by a complex microbial activity (Zeng *et al.*, 2013). In fermented foods, the types of microorganisms

involved in the fermentation process often play a crucial role in the safety, texture, colour, and flavour of product (Stergaard *et al.*, 1998). The metabolism of microbiota determines the composition and content of flavour-producing substances (Wolfe and Dutton, 2015; Wolfe *et al.*, 2014), and the composition of the microbiota is affected by environmental conditions (De Filippis *et al.*, 2015). Recently, the microbial community of traditional sour fish was analysed by the metagenomic analysis method (Yu *et al.*, 2017), and the results showed that *Lactobacillus* was the dominant bacteria (Riquelme *et al.*, 2015). However, studies conducted on fermented fish have not been reported yet. As unstable quality of flavour is the major problem in production of traditional fermented fish and modern industrial processing, understanding the structure of microbial community in fermented fish is a key for regulating and improving the quality of product.

Both microorganisms and endogenous enzymes promote the release of free fatty acids (FFA) in fermented fish, while microorganisms play a leading role in the production of flavour by various chemical reactions (Xu *et al.*, 2018). However, the mechanism of flavour formation during fermentation has not been understood completely, and more research is required to record the relationship between microbial community, flavour, and flavour precursors. Many studies have successfully studied the correlation between bacterial composition and flavour compounds (Pang *et al.*, 2018; Wang *et al.*, 2016; Zheng *et al.*, 2018). However, little research has been conducted on the relationship between bacterial succession and the volatile flavour components in fermented fish. In this study, free amino acids (FAAs), volatile flavour compounds, and microbial communities were analysed as fermentation progressed. The relationship between bacterial succession and the volatile flavour was analysed using the multivariate statistical analyses method, that is the bidirectional orthogonal partial least squares (O2PLS). These results would help to explore the core microbiota and flavour metabolites, and provide a technical reference for researchers in this field.

Materials and Methods

Study design and sampling

Alive common carp ($n = 140$) with an average weight of 0.5 ± 0.3 kg were obtained from Guizhou Yuhe Agricultural Products Co. Ltd (Guiyang, Guizhou, China) and killed according to the Guidelines for the Treatment of Animals and Experimental Animal Management Regulations issued by the Ministry of Science and Technology of the People's Republic of China. The fermented fish meat was prepared in accordance with the traditional method prescribed by the residents of Qiandongnan Prefecture

(Guizhou, China), with minor modifications. Briefly, the fish meat was pickled with 3% (w/w) standard kitchen salt at 4°C for 48 h and dried for 3 h at 50°C. After that, the fish meat was mixed with 30% (w/w) of fermented glutinous rice (Yonghui Supermarket, Guiyang, China) manually. After that, the prepared mixture was placed in 10-L oxygen-free jars (Farmer's market, Jinping, Guizhou, China) and fermented at ambient temperature (25°C) for 50 days. Three samples of fresh fish were taken (control) after pickled (0th), and on 10th, 20th, 30th, 40th and 50th day after fermentation; the samples taken on these time points were named CX, CTA, CTB, CTC, CTD, CTE, and CTF, respectively. All muscle samples were stored at -80°C for further analyses (conducted within 3 weeks).

Processing of sequence and community structure data

De-multiplexed and quality-filtered raw reads were analyzed using QIIME software (v.1.9.1). The representative operational taxonomic units (OTU) sequences were annotated using the database of QIIME-based wrapper of Ribosomal Database Project (RDP) classifier (v.2.2) (Wang *et al.*, 2007). Alpha diversity was calculated using the Hellinger distance between samples to reflect community diversity and richness. The relationships between samples were visualized according to principal component analysis (PCA) plots, which were calculated from the resulting distance matrices to compress dimensionality (Wang, 2012). Sequences of 97% identity were resolved at the species level (Ercolini *et al.*, 2012; Koyanagi, 2011). The representative sequences of all OTUs were uploaded to GenBank.

Analysis of amino acids content

Each fermented fish sample (2 g) was homogenised with 15 g/100-mL trichloroacetic acid (TCA) (Sigma Chemical Co., St. Louis, MO, USA) and diluted to 15 mL and incubated for 2 h. The mixture was centrifuged at 10,000 rpm for 15 min. After centrifugation, 5 mL of supernatant was removed to a new centrifuge tube, and the pH value was adjusted to 2.0 with 3 mol/L NaOH solution. The volume was then adjusted to 10 mL and the tube was shaken well. After filtration (0.22 µm), 400 µL of each sample was added into liquid phase vials (Sigma Chemical Co.). Finally, the content of amino acids was determined by the automatic amino acids analyzer. Each group was measured thrice in parallel.

Analysis of volatile compounds

The analysis of volatile compounds was conducted by employing the solid-phase microextraction (SPME)

combined with gas chromatography-mass spectrometry (GC-MS; GC6890-MS5975, Agilent Technologies, USA) on AHP-5MS column (30 m × 0.25 mm × 0.25-μm film thickness, Agilent Technologies) plus detection conditions with some modifications. Meat sample, 5 g, and 5-μL 0.18-g/mL NaCl were placed into a 15-mL headspace vial (Sigma Chemical Co.). Then the SPME fibre (65-μm PDMS/DVB, Supelco, USA) was put in the headspace of the sample vial at 60°C for 40 min. After extraction, the SPME fibre was immediately inserted into the GC-MS injector port at 250°C for 5 min.

Samples were analysed using the GC-MS software (TSQ Quantum XLS, Thermo Fisher Scientific, Waltham, MA, USA). The oven temperature was maintained at 40°C for 2 min, increased to 160°C at a rate of 4°C/min, and further to 250°C at a rate of 10°C/min, for 5 min. The inlet temperature was 250°C and the ionization energy was 70 eV. The temperature of the ion source was 230°C, with a corresponding interface temperature of 280°C and a detector temperature of 150°C. The model was put on full scanning, and the mass range was 35–350 *atomic mass* unit (amu). The data analysis was performed using GC-MS (TSQ Quantum XLS) software. Tentative identification of volatile compounds was determined by comparing mass spectra using NIST2005 and Wiley 7 databases (Hewlett–Packard, Palo Alto, CA, USA) and previously reported retention indices (RI). The retention indices of compounds were calculated after injection of an n-alkane series (C7–C26) with the same operating conditions. The 2,4,6-trimethylpyridine (0.5 mL, 100 ppm) was used as an internal standard, and the relative concentration of volatile compounds in the samples was calculated by comparing the peak area of each compound with that of internal standard, assuming equal responses for all compounds.

Data analysis

O2PLS was employed to reveal the relationship between bacteria at the genus level and different flavour components using SIMCA 14 (demo v.1.0.1) (Umetrics AB, Umeå, Sweden). It was carried out by integration of bacterial taxa (defined as X matrix) and flavours (defined as Y matrix) (Bylesjö *et al.*, 2007), which was evaluated by seven-fold cross-validation (Trygg, 2002). Terms with larger variable importance (VIP) value (>1) were most relevant for explaining Y variable. VIP (> 1) and correlation matrix ($|\rho| > 0.8$) were used to determine potential functional bacterial in fermented fish using the Microsoft® Excel, R software (v.2.14.1) and Cytoscape (v.2.8.3). PCA and hierarchical cluster analysis (HCA) were performed in SIMCA 14 software.

Results and Discussion

Changes and composition of bacterial communities at different stages of fermentation

We obtained 975,723 high-quality V4–V5 (16S) sequences from 21 fermented fish samples. The number of OTUs was 1,064 at CX stage, which dropped sharply to 356 at the end of fermentation. The diversity of bacteria (Chao 1 index) was highest in the CX sample. The Shannon and Simpson indices described the species number and distribution evenness respectively (Soto *et al.*, 2017). The fresh fish sample exhibited the highest bacterial richness. The likely reason is that the fish originated from a cultivating environment that had a high bacterial richness. As fermentation progressed, the bacterial diversity in samples tended to decrease, while bacterial richness was reduced starting from the 10th day of fermentation. It was probably that exogenous microorganisms were unable to adapt to the fermentation environment having a relatively high salt content and low pH (Ma *et al.*, 2016). Good's coverage corresponds to the sampling completeness; values >99% indicate that a sufficient bacterial diversity was captured by the sampling regime.

On the basis of OTUs categorised at the genus level, *Staphylococcus* was the most prominent bacterial genus (20.76%) at the CX stage, although its abundance decreased during subsequent stages. This was likely due to its poor acid tolerance, but some studies had reported presence of *Staphylococcus* in fermented seafood (Zhang *et al.*, 2015). As fermentation continued, the most abundant genera were *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Enterococcus*, and a few others. *Lactococcus* was the most dominant genus from CTC to CTF stages, which suggested its central role in the fermentation process. A similar observation was made by Zang *et al.* (2018). *Lactobacillus* and *Leuconostoc* were first observed at the CX stage; then their abundance increased with the progress of fermentation. The increased amount of metabolites of probiotic *Leuconostoc* and *Lactobacillus* could have influenced the growth of other bacteria (Ruiz-Moyano *et al.*, 2011). *Enterococcus* was found at the CTB stage and its abundance decreased with the fermentation process.

On the basis of dynamic changes that took place in bacterial community during the fermentation process, we divided the stages of fermentation into two parts: stage 1, comprising the first three experimental stages: CTX, CTA, and CTB; and stage 2, comprising the latter four experimental stages: CTC, CTD, CTE, and CTF. The bacterial profile similarity was evaluated using the distance between the sample points of PCA diagram. We observed that the same stage samples clustered together.

In the first direction (PC1), differences between different stages of fermentation were more obvious, accounting for 84.9% of variability (Figure 1). Previous studies have reported that the bacterial community was slightly different during the acetic acid fermentation stage (Nie *et al.*, 2017).

Analysis of flavour compounds in fermented fish

During fermentation, 124 flavour-associated compounds were detected, which included 17 FAAs and 107 volatile flavours (VFs). The total content of FAAs was less than 50 mg/100 g at the CX stage; then it increased, peaked at the CTC stage, and finally it decreased to 1,525.92 mg/100 g at CTF stage (50th day) (Table 1). Concentrations of aspartic acid (Asp), glycine (Gly), methionine (Met), leucine (Leu), and phenylalanine (Phe) exhibited trends comparable to that of the total FAAs. The content of total FAAs reached the maximum value between 40th and 50th day, but concentrations of lysine (Lys), histidine (His) and proline (Pro) reached the highest levels on 20th day (Table 1). The volatile flavours were divided into eight categories: 29 alcohols, 7 acids, 13 esters, 11 ketones, 20 aldehydes, 17 alkanes, 6 aromatics, and 4 others.

Considering the role of amino acids in development of flavours, it is important to determine their release during

meat fermentation (Beriaín *et al.*, 2000). In our study, the content of free amino acids in fermented samples was higher than in fresh samples. Quijada *et al.* (2017) reported that *Lactobacillus* produces organic acids that could be metabolised into amino acids, and *Lactobacillus* is abundantly found in fermented than in fresh samples.

The volatile flavour compounds increased during ripening, which indicated that microbial activity in fermented fish samples was higher than in fresh samples. Multiple studies have observed increased content of FAAs in fermented foods (Bolumar *et al.*, 2001; Casaburi *et al.*, 2007; Yin *et al.*, 2002a, 2012b). FAAs are closely related to the development of flavour in fermented products (Benito *et al.*, 2005). Kuda *et al.* (2009) found that main amino acids in aji-no-susu (a traditional fermented fish with rice produced in the Noto Peninsula, Japan) were Leu, glutamic acid (Glu), alanine (Ala), Lys, and threonine (Thr). Therefore, FAAs promote the development of specific flavour components in fermented fish.

As fermentation progressed, the total content of volatile components increased gradually: the number of volatile components ranged from 38 at the CX stage to 84 at the CTF stage. The PCA of volatile flavours indicated that the samples were in the same cluster at the initial stages (days 0 to 20), whereas the later stages have samples of the same cluster (days 25 to 35).

Table 1. The amino acid content of fermented fish at different time points.

Amino acid	CX	CTA	CTB	CTC	CTD	CTE	CTF
Asp	—	7.17 ± 0.41 ^a	28.70 ± 0.82 ^b	82.54 ± 6.77 ^c	131.10 ± 7.27 ^e	165.09 ± 5.64 ^f	116.59 ± 4.12 ^d
Thr	7.64 ± 0.48 ^a	17.36 ± 0.32 ^a	32.75 ± 1.10 ^b	101.35 ± 1.42 ^c	163.93 ± 11.59 ^e	147.66 ± 1.85 ^d	107.28 ± 1.26 ^c
Ser	5.67 ± 0.14 ^a	14.10 ± 0.95 ^b	17.69 ± 0.64 ^c	—	—	—	—
Glu	—	20.78 ± 1.43 ^a	79.96 ± 2.78 ^c	101.51 ± 4.21 ^d	106.85 ± 2.99 ^e	27.49 ± 0.81 ^b	28.35 ± 0.18 ^b
Gly	6.34 ± 0.11 ^a	16.26 ± 1.01 ^b	20.13 ± 0.67 ^b	51.23 ± 1.54 ^c	93.33 ± 5.74 ^d	122.50 ± 1.13 ^e	121.74 ± 0.58 ^e
Ala	11.77 ± 0.62 ^a	21.52 ± 0.94 ^a	54.12 ± 1.23 ^b	118.19 ± 9.31 ^c	213.41 ± 14.18 ^d	214.17 ± 5.36 ^e	361.29 ± 4.13 ^f
Cys	—	4.99 ± 0.16 ^a	14.78 ± 0.36 ^b	29.10 ± 1.07 ^c	61.83 ± 2.29 ^d	129.89 ± 1.40 ^f	171.89 ± 5.27 ^f
Val	—	8.71 ± 0.53 ^a	28.33 ± 1.19 ^b	56.76 ± 1.59 ^c	101.34 ± 3.81 ^d	186.13 ± 9.16 ^e	216.51 ± 0.61 ^f
Met	—	7.25 ± 0.22 ^a	20.32 ± 0.78 ^b	41.67 ± 1.24 ^c	69.12 ± 1.39 ^d	86.46 ± 0.64 ^f	82.68 ± 0.39 ^f
Ile	—	7.41 ± 0.29 ^a	19.18 ± 0.57 ^b	41.88 ± 2.60 ^c	72.83 ± 3.74 ^d	113.17 ± 1.38 ^f	118.34 ± 1.07 ^f
Leu	—	12.49 ± 0.14 ^a	51.74 ± 2.03 ^b	122.88 ± 9.97 ^c	224.25 ± 15.78 ^d	295.65 ± 11.42 ^f	284.50 ± 1.56 ^f
Tyr	—	10.18 ± 1.82 ^a	—	—	—	—	—
Phe	6.59 ± 0.08 ^a	18.36 ± 0.93 ^b	18.94 ± 0.27 ^b	41.91 ± 1.97 ^c	105.23 ± 1.93 ^d	129.53 ± 3.96 ^f	123.70 ± 1.34 ^f
Lys	—	18.55 ± 0.38 ^a	74.09 ± 3.12 ^c	186.17 ± 20.21 ^d	44.02 ± 1.17 ^b	16.86 ± 0.53 ^a	6.39 ± 0.17 ^a
His	7.11 ± 0.44 ^a	33.32 ± 1.42 ^b	60.64 ± 0.97 ^c	91.76 ± 1.18 ^f	83.06 ± 2.54 ^f	64.88 ± 0.54 ^d	64.52 ± 0.04 ^d
Arg	—	18.08 ± 17.21 ^a	—	—	—	—	—
Pro	2.53 ± 0.10 ^a	9.84 ± 0.62 ^d	18.01 ± 0.60 ^f	24.17 ± 1.50 ^g	4.76 ± 0.38 ^b	12.42 ± 0.61 ^f	6.64 ± 0.21 ^c
Total	47.65	246.37	539.38	1091.12	1475.06	1711.9	1525.92

Note: Mean values with different superscript letters within the same row indicate a significant difference at $P < 0.05$.

Figure 2. (A) PCA analysis of flavours during the fermentation process. (B) The biplot superimposed the scores and loadings of PCA analysis based on the correlation scaling method. R2VX represents the fraction of X variation modelled in the component. p(corr) and t(corr) is a combined vector, p(corr) represents loading p scaled as a correlation coefficient between X and t; t(corr) represents score t scaled as a correlation coefficient resulting in all points falling inside the circle with radius 1.

Figure 2 establishes that the contents and categories of volatile flavour compounds increased during the fermentation process. The biplot of O2PLS in Figure 2 reveals detailed characteristics of volatile flavour compounds at different time points during fermentation. Scores and

loadings suggest that hexadecane, acetophenone and heptanal were highly correlated at CX stage; octadecane, serine (Ser) and arginine (Arg) were highly correlated at CTA stage; camphor and styrene were highly correlated at CTB stage; cedrol was highly correlated at CTC stage;

7-hexadecenal and octanoic acid were highly correlated at CTD stage; propanoic acid and 2-heptanone were highly correlated at CTE stage; and 3-pentanol, acetic acid, hexanoic acid, and Asp were highly correlated at CTF stage.

Correlation between bacteria and flavours during the fermentation process

The relationship between bacteria and flavours during the fermentation process was analyzed by the O2PLS method: R2 and Q2 values of the model were 0.978 and 0.521, respectively (Table 2). When R2 and Q2 values were >0.5, the model is considered good (Dong *et al.*, 2015); therefore, these results suggest that the O2PLS method fitted well for analysis. The first two predictive components were significant at cross-validation, accounting for 97.8% of R2 (cum) and 93.4% of Q2 (cum) of this model. The VIP (pred) vector suggested that a total of 107 bacterial genera (VIP > 1) (Figure 3A) had important effects on flavours.

Rummeliibacillus, *Ralstonia* and *Pseudoxanthomonas* were the major bacterial taxa associated with the generation of flavours during fermentation. Based on the correlation coefficient between flavours and microbiota, a total of 59 bacterial genera were highly correlated ($|\rho| > 0.8$) with two flavour sets, among which a total of 27 bacteria were correlated with volatile flavours (light red circles on the right side of Figure 3B), and 22 bacteria were correlated with amino acids (light green circles on the right side of Figure 3B). *Lactobacillus* and *Veillonella* had the maximum number of strongly correlated flavours (19 and 13, respectively) ($|\rho| > 0.8$). As for amino acids, Ala, Leu and valine (Val) were major contributors to the flavour of fermented fish. Leu, which provides umami taste to fermented fish, was correlated with three genera ($|\rho| > 0.8$). Ala, which provides sweet taste to fermented fish, was also highly correlated with three bacterial genera ($|\rho| > 0.8$). Val, which provides sweet and bitter taste to fermented fish, was highly correlated with two bacterial genera ($|\rho| > 0.8$).

A total of 27 genera were correlated with 45 volatile flavours ($|\rho| > 0.8$), among which *Psychrobacter*, *Lactobacillus*, *Rhodococcus* and *Corynebacterium* were correlated with 11, 9, 9 and 7 volatile flavours ($|\rho| > 0.8$), respectively. *Lactobacillus* was positively correlated with most of the flavours; its correlation coefficients with

phenylethyl alcohol, decanoic acid, ethyl ester, hexadecanoic acid, ethyl ester, and methyl salicylate were >0.9. Some bacterial genera were also negatively correlated with some flavours; for example, the correlation coefficient between *Lactobacillus* and 1-Penten-3-ol was -0.8, and both *Psychrobacter* and *Rhodococcus* were negatively correlated with most of the flavours (Figure 3B).

Few studies have examined the relationship between bacteria and flavours in traditional fermented foods (Carpino *et al.*, 2017). In our study, the O2PLS method was merged with linear regression analysis to combine bacterial and flavour dataset to determine relationship between bacteria and flavours in the fermentation process. A total of 59 bacterial genera were strongly correlated with most fermented fish flavours across all seven time points, suggesting that bacteria could be the main producer of flavours.

For example, *Lactobacillus*, *Leuconostoc* and *Lactococcus* had a strong positive correlation with 29 flavours, whereas *Pseudomonas* and *Erwinia* had a significant positive correlation with 15 flavours during fish fermentation. These bacteria may contribute to the formation of flavours, but this could be a new topic that needs to be studied in the future. Interestingly, we found that *Lactobacillus* was the most prominent bacteria across all seven time points. This genus partakes in the formation of acetic acid, but it is rarely a dominant bacterial strain (Aldrete-Tapia *et al.*, 2014; Escobar-Zepeda *et al.*, 2016). This indicates that fermented fish is rather unique in the aspect of composition of bacterial community. Regarding the functional relationship between bacterial community and flavour components, the bacteria and flavour depicted a very high correlation. *Lactobacillus* was the main factor to promote the formation of flavour. It has been reported that *Lactobacillus*, yeasts, and *Staphylococcus* are the main microorganisms associated with the fermentation and ripening of meat products (Ojha *et al.*, 2015). *Lactobacillus* promotes the formation of acids and plays an important role in the fermentation process (Ozogul *et al.*, 2017). The rapid growth of *Lactobacillus* could inhibit the growth of other microorganisms, thereby ensuring the safety of fermented meat products (Ojha *et al.*, 2015). *Staphylococcus* is often used as a starter and added to fermented meat products together with *Lactobacillus* (Martin *et al.*, 2007; Ojha *et al.*, 2015). Esters, alcohols, acids, aldehydes, and ketones contribute to the unique flavours of fermented fish (Gao *et al.*, 2016a; Zeng *et al.*, 2016). The results of this study indicate that there is a high correlation between bacteria and flavour, which is similar to the results of the study conducted by Zheng *et al.* (2018).

However, some questions remain unresolved. Firstly, because a wide variety of bacteria produce flavour

Table 2. Model parameters

Model	Type	A	N	R2X (cum)	Q2 (cum)
M1	PCA-X	6	21	0.978	0.521

Figure 3. Correlation analyses between bacteria and flavours during the fermentation process inferred using the O2PLS modelling. (A) VIP(pred) plot of important microbiota (VIP(pred) > 1.0). (B) Correlation network between microbial genera and flavours during the fermentation process. The left-side circles represent volatile flavours (squares) and amino acids (hexagons) correlated with flavours ($|\rho| > 0.8$). The right-side circles represent the flavours correlated with microbiota ($|\rho| > 0.8$). The red dash lines linking the circles represent a positive correlation, while the purple dash lines represent a negative correlation between microbiota and flavours.

components through highly interconnected metabolic pathways, it was difficult to determine the flavour components that were produced by certain group of bacteria based on the current data. Secondly, the composition of fungal colonies must also be considered.

Conclusions and Prospects

During fermentation, a significantly positive correlation was established between *Lactobacillus* and *Psychrobacter*, which were dominant bacteria. However, *Pseudomonas* had a significantly negative correlation with various flavours. Therefore, different bacteria contribute to different flavours during different stages of fermentation. This implies that the quality of fermented fish is dependent on how well-balanced are the bacteria. The exploration of bacterial dynamics during fish fermentation provides valuable information for understanding the complete ecology of fish fermentation systems and provides guidelines for quality control.

This was the first study on the bacterial and flavour dynamics of fermented fish. Hence, the future studies on relationships among bacterial communities and metabolites could lead to a better understanding of the fish fermentation mechanics, thereby ensuring high-quality and production safety of fermented fish. However, the mechanism of microflora-driven flavour production during the fermentation of fish also needs to be explored in the future.

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