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Bacterial community diversity of traditional fermented vegetables in China

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ABSTRACT

Bacterial community composition influences the formation of flavourful substances in fermented foods. However, the bacterial structure of fermented vegetables from different origins has scarcely been reported. Here, 10 different fermented vegetables of Chinese origin were collected to explore the bacterial community diversity using 16S rDNA sequencing. Of these samples, the number of identified operational taxonomic units (OTUs) ranged from 107 to 290, which could be categorized into 12 phyla, 21 classes, 52 orders, 97 families, 223 genera and 348 species. Firmicutes and Proteobacteria were the top two dominant phyla with a relative abundance of 88.2–99.3% of the total OTUs. Meanwhile, *Lactobacillus alimentarius*, L. *versmoldensis*, *Bacillus* and unclassified *Weissella* spp. were detected as the predominant Firmicutes bacteria, while *Chromohalobacter japonicus*, *Halomonas jeotgali*, *Pediococcus* and unclassified *Vibrio* spp. were the most abundant proteus bacteria. Hierarchical cluster and principal component analysis indicated that the 10 samples could be grouped into three groups, with the main two components explaining 49.1 and 28.6% of the total variance. These results indicated a high level of diversity in the bacterial community structure in traditional fermented vegetables and demonstrated the possible influences of manufacturing location and conditions, and/or the type of raw material, on bacterial diversity.

1. Introduction

Fermentation is an ancient and widely used processing technique used to preserve fresh ingredients in order to prolong shelf life and increase nutritive value. It facilitates the development of a diversity of flavours, aromas and textures in food substrates (Liu, Han, & Zhou, 2011). Fermented vegetables, like other types of fermented products, are popular and traditional edible foods in Asia, and are comprised of a variety of vegetables such as mustard (Chao, Kudo, Tsai, & Watanabe, 2012; Chao, Wu, Watanabe, & Tsai, 2009), cucumber (Chen et al., 2012; Tamang et al., 2005), cabbage (Chang et al., 2008; Cho et al., 2006; Nam, Chang, Kim, Roh, & Bae, 2009), radish (Tamang & Sarkar, 1993; Tamang et al., 2005), pepper (Xiao et al., 2010) and bamboo shoots (Jeyaram, Romi, Singh, Devi, & Devi, 2010; Tamang & Tamang, 2009), developed using different manufacturing processes. Unique flavours and substances are produced during fermentation, which stimulates the appetite and enhances digestibility, contributing to the popularity of fermented foods. Enhancing the quality of fermented vegetables may lead to increased nutritional value and health-benefits to consumers.

Presently, numerous anti-microbial substances, sugar polymers, sweeteners, aromatic compounds, essential fatty acids, vitamins, useful enzymes and other probiotic properties have been found to be closely associated with microorganism populations in fermented foods (Leroy & De Vuyst, 2004; Liu et al., 2011). In recognition of the significance of microorganisms in quality formation of fermented foods, extensive studies on the filtering and physicochemical properties of functional microorganisms spontaneously occurring in fermentation have been reported. A number of genera and species of microorganisms in different fermented food products such as fermented milk, vegetables, cereals, legumes, meats and fish have been isolated (Tamang, Watanabe, & Holzapfel, 2016). Although numerous fermented vegetables are produced globally, the types of microorganisms occurring during the fermentation process appears to depend on the manufacturing method, season and geographical area (Nguyen et al., 2013). Of the microorganisms

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present in fermented vegetables, lactic acid bacteria (LAB) are frequently identified as the predominant bacterial genera throughout the fermentation process, including *Lactobacillus*, *Pediococcus*, *Weissella* and *Leuconostoc* (Chao et al., 2009; Nguyen et al., 2013). Based on their individual functions during fermentation, these various LAB have been engineered for the production of L-alanine, vitamins, exopolysaccharides, bacteriocins and aromatic compounds (Alexandraki, Tsakalidou, Papadimitriou, & Holzapfel, 2013) via metabolizing the chemical components of raw materials (Nguyen et al., 2013).

It has been demonstrated that the leading types of LAB isolated in fermented vegetables differ significantly between regions and with respect to the raw materials used. For example, dominant LAB in Philippine fermented mustard products include *Lactobacillus brevis* and *Pediococcus cerevisiae* (Rhee, Lee, & Lee, 2011), while only *L. brevis* was dominant in Thai fermented mustard products (Rhee et al., 2011). Conversely, *L. crustorum*, *L. farciminis* and *L. mindensis* were isolated as important microorganisms in Taiwanese mustard products (Chao et al., 2012). Tamang and co-workers reported the major LAB representative involved in fermented radish to be *L. brevis* (Tamang et al., 2005). However, *L. sakei*, *L. plantarum*, *L. brevis*, *Leuconostoc mesenteroides* and *P. cerevisiae* were found to be single or multiple dominated LAB in fermented radish from China, Korea and Japan (Alexandraki et al., 2013; Jiang et al., 2012). Furthermore, dynamic changes in the proportion of the LAB *L. curvatus*, *L. plantarum*, *L. mesenteroides* and *Weissella confusa* have been identified in raw red and yellow pepper fruits, and accordingly, *L. plantarum* PE21, *L. curvatus* PE4 and *W. confusa* PE36 have been selected in the mixed starter culture for pepper fermentation (Di Cagno et al., 2009). Despite a growing understanding of the ecology of microorganisms of many fermented vegetables, the selected fermented samples in these studies were too limited to reveal the variation and particularity of the bacteria between areas.

To our knowledge, detailed investigations of bacterial community variation in Chinese fermented vegetables remain scarce. It is therefore necessary to acquire a better and more accurate understanding of the microorganism community in traditional fermented vegetables derived from various regions. The objective of the present study was to define the diversity and variation of the native bacterial communities among 10 conventional fermented vegetables originating from eight regions in southwest and southeast China using 16S rRNA sequencing. The results should improve our understanding of the differences in bacterial diversity between regions, thereby helping us to select optimal and appropriate functional bacteria for manufacturing high-quality and distinctive fermented vegetable products.

2. Materials and methods

2.1. Materials

Ten different traditional fermented vegetables were collected, including seven tuber mustards (*Brassica juncea* var. tumida), one potherb mustard (*Brassica juncea* var. crispifolia), one radish (*Raphanus sativus* L.) and one pepper (*Capsicum annuum* L.) from 10 independent manufacturers in southeast and southwest China (Table 1). The tuber mustards and pepper were fermented in brine, while the radish and potherb mustard were fermented in salt (Table 1). The bacteria from the fermented vegetables were collected in triplicate and kept on dry ice and later stored at −80 °C once all materials had been obtained.

2.2. DNA extraction and PCR amplification for 16S rDNA

Genomic DNA was extracted from each bacterial sample (combination of three replicates from the same location) using the E.Z.N.A.® Soil DNA Extraction Kit (Omega Bio-tek, Norcross, GA, U.S.). Two universal primers 515F (5′-barcode- GTGCCAGC MGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′), where the barcode was an eight-base sequence unique to each sample, were used in PCR to amplify the V4-V5 region of the bacteria 16S ribosomal RNA gene. The PCRs were carried out in 20 μL volumes with 10 ng of template DNA, 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.4 μL of FastPfu polymerase and 0.8 μL of each 5 μM primer. The PCR amplification was run under following conditions: 1) pre-denature at 95 °C for 2 min, and 2) followed by 25 cycles of denaturing at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, 3), and a final extension at 72 °C for 5 min.

Table 1
Information of fermented vegetable samples used in this study.

Code	Vegetable	Fermentation method	Production location
PMCXZ	Mustard tuber	Brine	Cixi, Zhejiang
PMFLC1	Mustard tuber	Brine	Fuling, Chongqing
PMFLC2	Mustard tuber	Brine	Fuling, Chongqing
PMMSS1	Mustard tuber	Brine	Meishan, Sichuan
PMSYZ	Mustard tuber	Brine	Shangyu, Zhejiang
PMTXZ	Mustard tuber	Brine	Tongxiang, Zhejiang
PMYYZ	Mustard tuber	Brine	Yuyao, Zhejiang
PPMNBZ	Mustard leaf	Salted	Ningbo, Zhejiang
PPMSS1	Pepper	Brine	Meishan, Sichuan
PRXSZ	Radish	Salted	Xiaoshan, Zhejiang

The PCR products were detected by 2% denaturing agarose gels, after which the 16S rRNA gene amplicons were extracted from the gels and purified with a AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) and quantified with QuantiFluor™ -ST (Promega, U.S.). The purified 16S rDNA gene amplicons were paired-end sequenced on an Illumina MiSeq platform at Majorbio Company (Shanghai, China).

2.3. Sequence analysis

The raw sequence reads were deposited into the NCBI Sequence Read Archive (SRA) database. Raw fastq files were de-multiplexed and quality-filtered using QIIME (version 1.17) with the following criteria: 1) 250 bp reads at any site that received an average quality score <20 over a 10 bp sliding window were truncated, and the truncated reads that were shorter than 50 bp were discarded. 2) Using exact barcode matching, two nucleotide mismatches in primer matching and reads containing ambiguous characters were removed. 3) The sequences that overlapped more than 10 bp based on their overlap sequence were merged, while those reads that could not be assembled were discarded using FLASH (Magoč & Salzberg, 2011).

Operational taxonomic units (OTUs) were clustered at a 3% cut-off using UPARSE (version 7.1 http://drive5.com/uparse/) and chimeric sequences were identified and removed with UCHIME. The phylogenetic affiliation of each 16S rRNA gene sequence was analysed by the RDP Classifier (http://rdp.cme.msu.edu/), while the Silva (SSU115) 16S rRNA database was referenced with a confidence threshold of 0.7. The α-diversity of 16S rRNA gene sequence including the ACE estimator, Chao estimator, Shannon and Simpson indexes were analysed using Mothur (version 1.30.1, http://www.mothur.org/wiki/Schloss_SOP#Alpha_diversity; Schloss, Gevers, & Westcott, 2011).

2.4. Nucleotide sequence accession numbers

The sequences of V4-V5 16S rRNA genes were deposited in the