Culture-independent analysis of the bacterial community in Chinese fermented vegetables and genomic analysis of lactic acid bacteria

Jianming Zhang¹ · Hye Seon Song² · Chengcheng Zhang¹ · Yeon Bee Kim² · Seong Woon Roh² · Daqun Liu¹

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Abstract
Six different fermented vegetables were collected from Zhejiang Province, China, to explore the associated bacterial community using a high-throughput sequencing platform. A total of 24 phyla, 274 families and 569 genera were identified from 6 samples. Firmicutes and Proteobacteria were the main phyla in all of the samples. Brevibacterium was the major genus in Xiaoshan pickled radish. Lactobacillus-related genera and Vibrio were the major genera in fermented potherb mustard and its brine. Enterobacter and Cobetia were the major genera in fermented radish and its brine. Chromohalobacter was the major genus in the tuber mustard. These results indicated clear differences were there between the bacterial genera present in Xiaoshan pickled radish, fermented potherb mustard, fermented radish, and tuber mustard. This demonstrated the possible influences of raw materials and manufacturing processes. Furthermore, a large number of lactic acid bacteria were isolated and identified by culture-dependent and 16S rRNA gene sequence analysis, which accounted for more than 68% of all the isolates. In addition, whole-genome analysis of Levilactobacillus suantsaii, Latilactobacillus sakei subsp. sakei, and Weissella cibaria showed that they had large numbers of genes associated with carbohydrate metabolism. This may explain why these three bacterial strains can grow in fermented vegetable environments.

Keywords Culture-independent analysis · Bacterial community · Chinese fermented vegetables · Genomic analysis

Introduction
Thousands of years ago, the ancestors of the Chinese people began fermenting vegetables to extend shelf-life (Guan et al. 2020; Zhang et al. 2021). Most Chinese people continue to make fermented foods using perishable and seasonal vegetables, such as radish, cabbage, mustard, and cucumber (Liang et al. 2018; Zhai et al. 2018; Liu et al. 2019a; Rao et al. 2020). The purpose of fermenting vegetables is not only to prolong shelf-life, but also to enhance nutrition and improve organoleptic characteristics. There are various fermented vegetables in China, such as paocai in Southwest China, and serofluid dish in Northwest China (Li et al. 2017; Liu et al. 2019b). Zhejiang Province, which is located in Southeast China, has a unique environment with rich topography and abundant rainfall. Due to this unique climate, local people particularly enjoy the fermented vegetables produced in the region. Xiaoshan pickled radish, fermented potherb mustard, and meigan cai are distinctive traditionally fermented foods that have been consumed for thousands of years in Zhejiang Province (Guan et al. 2020; Zhang et al. 2019). Despite the geographic and climatic consistency, the flavor, taste, and texture of these fermented vegetables differ. The changes in the texture and flavor of the fermented vegetables are dependent on the microorganisms present during the fermentation process. This is because bacteria contribute to the flavor formation and acidification of the foods (Guan et al. 2020). Therefore, various measures have been employed to elucidate the structure and composition of the bacterial community in the fermented vegetables.
Over recent decades, culture-independent methods have been successfully employed to analyze the microbial community in fermented vegetables. For example, denaturing gradient gel electrophoresis has been used to study the microbial ecology of suancai and serofluid dish in China (Wu et al. 2015; Zhang et al. 2018; Zhou et al. 2018). Real-time quantitative PCR has also been used to detect both bacteria and yeast in Chinese paocai (Xiong et al. 2019). At present, high-throughput sequencing (HTS) is the most common method to reveal the major bacterial community members in fermented vegetables. HTS can provide more detailed information and deeper insight into the microbial community compared to conventional molecular methods (Chen et al. 2019; Xiao et al. 2020). Nevertheless, HTS is based on community analysis of environmental DNA, and so pure cultures cannot be isolated and their physiological potentiality remains unknown. Therefore, some studies have combined culture-independent and culture-dependent techniques to analyze the bacterial community and obtain pure cultures (Lu et al. 2020; Wang et al. 2020).

In this study, six fermented vegetables were collected from Zhejiang. Culture-independent HTS and a culture-dependent method were used in combination to investigate the bacterial community and isolate specific strains from the six fermented vegetables. Furthermore, the genomes of *Levilactobacillus suantsaii* (L. suantsaiii) CBA3634, *Latilactobacillus sakei* subsp. sakei (L. sakei subsp. sakei) CBA3635, and *Weissella cibaria* (W. cibaria) CBA3636, which were isolated from fermented potherb mustard and its brine, were sequenced using the PacBio and Illumina platforms. This work aimed to deepen the understanding of the bacterial community in the fermented vegetables of Zhejiang and isolate and elucidate key features of the dominant lactic acid bacteria (LAB) responsible for vegetable fermentation. The results of this study will be helpful for stabilizing and enhancing the characteristics of fermented vegetables of Zhejiang.

**Materials and methods**

**Sample collection**

Samples were collected from six traditional fermented vegetables (C1–C6) from Zhejiang Province in 2018 (Fig.S1). Xiaoshan pickled radish (C1) was obtained from a factory in Hangzhou and the manufacturing process was as follows. A fresh radish was washed, cut evenly into strips, and air dried for 3–5 days. The radish was then mixed with a 6–7% (6–7 g/100 g) concentration of sodium chloride and stored at room temperature in the dark for approximately 1 year. Fermented potherb mustard (*Brassica juncea* var. *multiceps*; C2) and the brine of fermented potherb mustard (C6) were obtained from the factory in Hangzhou. The potherb mustard was pickled in a 16–18% concentration of sodium chloride and had been stored at room temperature in the dark for approximately 1 year. Fermented radish (C3) and its brine (C5) were obtained from the factory in Hangzhou. The fresh radish was washed and pickled in an 18–20% (18–20 g/100 g) concentration of sodium chloride. Then, the radish was stored at room temperature in the dark for approximately 1 year. Tuber mustard (*Brassica juncea* var. *tumida*; C4) was obtained from the factory in Hangzhou. Tuber mustard was pickled in a 14–17% concentration of sodium chloride and had been stored at room temperature in the dark for approximately one year.

**Culture-independent HTS analysis of the bacterial community**

Total genomic DNA was extracted from the six samples using a FastDNA SPIN kit for soil (MP Biomedicals, Solon, OH, USA), according to the manufacturer’s instructions. The amplicon library was prepared using a two-step PCR approach. In the first step, the hypervariable V3–V4 region of the bacterial 16S rRNA gene was amplified using the primer pair 341F and 805R (Fadrosh et al. 2014). In the second step, the dual index barcodes, i5 and i7, of Illumina Nextera were attached to each sample. The quantity and quality of the constructed library were determined using a Quant-iT PicoGreen dsDNA Assay kit (Invitrogen, Waltham, MA, USA) and an Agilent 2100 Bioanalyzer System (Agilent Technologies, Santa Clara, CA). The final library products were sequenced using an Illumina MiSeq platform (Illumina, San Diego, CA, USA) according to Illumina’s standard protocol. Bioinformatic analyses were performed using the free online Majorbio Cloud Platform (www.majorbio.com), provided by Shanghai Majorbio Bio-pharm Technology Co. Ltd, according to the method described by Huang et al. (2020).

**Cultivation of LAB**

De Man, Rogosa, and Sharpe (MRS, peptone 10.0 g/L; ‘Lab-Lemco’ powder 8.0 g/L; yeast extract 4.0 g/L; glucose 20.0 g/L; sorbitan mono-oleate 1 mL/L; di-potassium hydrogen phosphate 2.0 g/L; sodium acetate 3H2O 5.0 g/L; tri-ammonium citrate 2.0 g/L; magnesium sulfate 7H2O 0.2 g/L; manganese sulfate 4H2O 0.05 g/L) and Rogosa SL media (tryptone 10.0 g/L; yeast extract 5.0 g/L, dextrose 10.0 g/L, sucrose 5.0 g/L, arabinose 5.0 g/L, sodium Acetate 15.0 g/L, ammonium citrate 2.0 g/L, monopotassium phosphate 6.0 g/L, magnesium sulfate 0.57 g/L, manganese sulfate 0.12 g/L, ferrous sulfate 0.03 g/L, tween 80 1.0 g/L) were used to isolate cultivable LAB from all of the six fermented vegetable samples. The samples were serially diluted.
and plated on MRS and Rogosa SL agar media. Each plate was incubated in aerobic conditions at 30 °C for 48 h. After cultivation, 44 colonies were randomly picked and transferred several times until pure cultures were obtained. The 16S rRNA gene fragments of each isolate were amplified by colony PCR using the bacterial universal primer set 27F and 1492R. The PCR products were analyzed by agarose gel electrophoresis and sequenced using Sanger sequencing. The 16S rRNA gene sequences were identified using the EzBioCloud database (https://www.ezbiocloud.net/identify).

Genomic analysis of the isolated LAB

DNA extraction

Genomic DNA was extracted using an MG Genomic DNA Purification Kit (MGmed, Seoul, Korea) according to the manufacturer’s protocol. The quality and quantity of extracted genomic DNA were estimated using an Agilent 2100 Bioanalyzer (Agilent Technologies Inc., California, USA) and NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

Library construction and sequencing

Whole-genome sequencing of the isolates was performed using a combination of the PacBio RS II and Illumina HiSeq sequencing platforms. The Illumina data were used to evaluate the complexity of the genome. For the PacBio sequencing, 20 kb SMRTbell libraries were constructed using a standard method and sequenced using the PacBio RS II system with the P6 DNA polymerase and C4 chemistry. For the Illumina HiSeq sequencing, paired-end libraries were constructed for the isolates using a TruSeq Nano DNA kit (350 bp insert size) following the manufacturer’s recommendations and were sequenced using the Illumina HiSeq system.

Genome assembly and annotation

The sequencing reads obtained from the PacBio sequencer were de novo assembled using the hierarchical genome assembly process. After de novo assembly, the Illumina sequencing reads were integrated into the draft assemblies to obtain accurate genome sequences using the Pilon tool. The circular form of the contigs was confirmed using the UGENE software package (Komstantin et al. 2012).

Genome annotation was performed using Pathosystems Resource Integration Center database (PATRIC) and NCBI Prokaryotic Genome Annotation Pipeline (PGAP). The circular genome map was constructed using the CGview Server tool (http://cgview.ca/), based on the CDS annotated by Prokka (Anna et al. 2020). The predicted protein coding sequence (CDS) was assigned a K number using BlastKOALA and classified into a KEGG pathway using the Reconstruct Module tool in the KEGG Mapper (Kanehisa et al. 2016). The Prophage Hunter tool was used to predict prophage activity within the genome. The 16S rRNA gene from the genomes was used to construct a phylogenetic tree using MEGA7 according to the neighbor-joining method. The topology of the phylogenetic tree was evaluated with 1,000 bootstrap replications and values > 70% were indicated in the tree.

Results

HTS analysis of bacterial community structure

The number of effective sequence reads obtained from the six fermented vegetable samples ranged from 21,388 to 68,573. Based on a similarity threshold of 0.97, a total of 1,131 operational taxonomic units (OTUs) were identified from all the samples. Sixty-seven of these OTUs were present in all of the samples. In total, 130, 98, 147, 114, 64, and 68,573 OTUs were unique to the C1, C2, C3, C4, C5, and C6 samples, respectively (Fig. 1A). Species richness estimators (Table 1) were used to reflect the microbial phylotype richness levels. The results revealed that the C3 sample had the highest microbial diversity among the six samples, and rank-abundance curves (Fig. 1B) also illustrated this result.

A total of 24 phyla, 274 families, and 569 genera were identified in the 6 samples. At the phylum level, the major phyla were Firmicutes and Proteobacteria. Firmicutes was the most dominant phylum in the C1 and C2 samples, accounting for 34.48% and 81.97% of the total phyla, respectively. Further, Proteobacteria, the second most dominant phylum, accounted for 33.37% and 15.51% of the total phyla in the C1 and C2 samples, respectively. On the contrary, Proteobacteria was the most dominant phylum in C3, C4, C5, and C6, accounting for 68.56%, 56.73%, 67.92%, and 79.14% of the total phyla, respectively. Firmicutes was the second most dominant phylum in C3, C4, C5, and C6, accounting for 15.82%, 25.27%, 25.81%, and 8.29% of the total phyla, respectively. Firmicutes was the most dominant phylum in C1, C2, C3, C4, C5, and C6 samples, respectively. At the family level, Halomonadaceae was most abundant in the C4 (53.83%) and C5 (27.47%) samples. Lactobacillaceae was the most dominant family in C2 (73.73%) and Brevibacteriaceae was the most dominant family in C1. Enterobacteriaceae was the most dominant family in C3, and Beijerinckiaceae was the most dominant family in C6 (Fig. 2B).

At the genus level, Brevibacterium (17.39%) was the most abundant genus in the C1 sample, followed by Leuconostoc (13.94%), Lactobacillus-related genera (11.50%) (Zheng et al. 2020; Kim et al. 2021), Pseudomonas (6.29%), and Halomonas (6.19%). In C2, Lactobacillus-related genera
(73.72%) were the major genera observed, and this was followed by Weissella (7.46%) and Methylobacterium (4.72%). Enterobacter, Ralstonia, Lactobacillus-related genera, and Lactococcus were majority in C3, accounting, respectively, for 6.5%, 5.31%, 5.29%, and 5.15% of the total genera. In C4, Chromohalobacter, Halomonas, Halanaerobium, and Lactobacillus-related genera were the dominant genera, accounting for 27.68%, 21.08%, 16.13%, and 13.82%, respectively, of the total genera. In addition, Cobetia (27.10%), Lactobacillus-related genera (10.36%), Enterobacter (6.84%), Pseudoalteromonas (5.55%), and Lactococcus (5.42%) were highly abundant in the C5 sample. Vibrio (12.37%), Methylobacterium (12.33%), Pseudoalteromonas (10.78%), Pseudomonas (8.82%), Sphingomonas (6.52%), and Enterobacter (5.95%) were the major genera in the C6 sample (Fig. 2C).

### Table 1 The diversity indices of bacterial communities in the six samples

| Sample | ACE     | Chao1    | Coverage (%) |
|--------|---------|---------|--------------|
| C1     | 567.79  | 549.75  | 0.99         |
| C2     | 449.97  | 436.89  | 0.99         |
| C3     | 653.07  | 643.01  | 0.99         |
| C4     | 349.38  | 341.33  | 0.99         |
| C5     | 475.71  | 467.44  | 0.99         |
| C6     | 590.61  | 573.40  | 0.99         |

C1: Xiaoshan pickled radish; C2: fermented potherb mustard; C3: fermented radish; C4: tuber mustard; C5: fermented radish bring; C6: fermented potherb mustard bring

### Isolation and molecular identification of LAB strains

Several colonies on MRS or Rogosa SL plates were isolated and purified from the four samples (C1, C2, C5, and C6). Forty-four isolates were identified via 16S rRNA gene sequence analysis. From C1, eight Staphyloccocus (six Staphylococcus piscifermentans and two Staphylococcus nepalensis) and one Terribacillus (Terribacillus goriensis) were obtained. Four Companilactobacillus (three Companilactobacillus alimentarius and one Companilactobacillus versmoldensis), one Levilactobacillus (Levilactobacillus suantsaii) and two Bacillus (one Bacillus altitudinis and one Bacillus gossypii) were obtained from the C2 sample. Ten Latilactobacillus (five Latilactobacillus curvatus, five Latilactobacillus sakei subsp. sakei) and one Lactiplantibacillus (one Lactiplantibacillus pentosus) were obtained from the C5 sample. From C6, 3 Latilactobacillus (Latilactobacillus sakei subsp. sakei), 11 Leuconostoc (4 Leuconostoc mesenteroides subsp. mesenteroides, 5 Leuconostoc mesenteroides subsp. jonggajibkimchii, and 2 Leuconostoc carnosum), 2 Weissella (1 Weissella soli and 1 Weissella cibaria), and 1 Enterococcus (Enterococcus casselii) were obtained. Thirty of these were LAB strains, which accounted for more than 68% of all isolates. Furthermore, the representative LAB strains from the fermented foods, Levilactobacillus suantsaii (L. suantsaii) CBA3634, Latilactobacillus sakei subsp. sakei (L. sakei subsp. sakei) CBA3635, and Weissella cibaria (W. cibaria) CBA3636, were selected for whole-genome analysis (Table S1).
Fig. 2  Bacterial communities in the six samples at the phylum (A), family (B), and genus (C) level. C1: Xiaoshan pickled radish; C2: fermented potherb mustard; C3: fermented radish; C4: tuber mustard; C5: fermented radish bring; C6: fermented potherb mustard bring
Whole-genome analysis of *L. suantsaii*, *L. sakei* subsp. *sakei*, and *W. cibaria*

The genomes of the three strains, *L. suantsaii* CBA3634, *L. sakei* subsp. *sakei* CBA3635, and *W. cibaria* CBA3636, were sequenced using the PacBio sequencer and HiSeq platforms. The *L. suantsaii* CBA3634 genome consisted of a circular chromosome and five plasmids. The length of the genome was 2,661,801 bp with a GC content of 50.49% (Fig. 3A, Table 2). The genome contains 18 rRNA genes, 70 tRNA genes, and 2362 CDSs. Of the CDSs, 56.0% (1322 of 2362) were assigned protein functions and classified into 38 KEGG categories. The top three assigned KEGG categories were “global and overview maps,” “carbohydrate metabolism,” and “amino acid metabolism” (Fig. 3D). One active and two ambiguous prophage regions were predicted in the CBA3634 genome. The active prophage closely matched with the *Lactobacillus* phage LBR48, and the ambiguous prophages closely matched with the *Lactobacillus* phage LBR48 and the *Streptococcus* phage phiJH1301-2.

Table 2  General genome features of *L. suantsaii* CBA3634, *L. sakei* subsp. *sakei* CBA3635, and *W. cibaria* CBA3636

| Feature         | CBA3634  | CBA3635  | CBA3636  |
|-----------------|----------|----------|----------|
| Genome size     | 2,661,801| 2,006,110| 2,423,709|
| GC content (%)  | 50.49    | 41.13    | 44.99    |
| rRNAs           | 18       | 21       | 28       |
| tRNAs           | 70       | 66       | 89       |
| Predicted genes | 2362     | 2002     | 2165     |
The complete genome of *L. sakei* subsp. *sakei* CBA3635 was composed of a single circular chromosome of 2,006,110 bp with a GC content of 41.13%, and one circular plasmid of 56,165 bp with a GC content of 41.11%. There were 2,002 CDSs, 21 rRNA operons, and 66 tRNAs in the genome (Fig. 3B and Table 2). The 1,130 CDSs of *L. sakei* subsp. *sakei* CBA3635 were assigned protein functions and classified into 37 KEGG categories, as shown in Fig. 3D. With regard to the KEGG categories of strain CBA3635, “global and overview maps” (528 genes), “carbohydrate metabolism” (174 genes), and “translation” (81 genes) were assigned at high proportions. Two active and two ambiguous prophage regions were predicted in the CBA3635 genome. The active prophages closely matched with *Lactobacillus* phage PL-1 and *Staphylococcus* phage IME1361_01. The ambiguous prophages closely matched with *Lactobacillus* prophage Lj965 and *Staphylococcus* phage SPbeta-like.

The complete genome of *W. cibaria* CBA3636 included one circular chromosome and two circular plasmids. The total length was 2,423,709 bp with a GC content of 44.99% (Fig. 3C and Table 2). The genome contained 2,165 CDSs, 28 rRNA genes, and 89 tRNAs. Of the CDSs, 56.9% (1,232 of 2,165) were assigned protein functions and were divided into 38 KEGG categories. The highest proportion of these genes was assigned to the category of “global and overview maps” (597 genes), followed by “carbohydrate metabolism” (174 genes) and “translation” (81 genes) were assigned at high proportions. Two active and two ambiguous prophage regions were predicted in the CBA3635 genome. The active prophages closely matched with *Lactobacillus* phage phi-SsUD.1. The maximum likelihood phylogenetic tree was constructed based on the 16S rRNA sequences of *L. suantsaii* CBA3634, *L. sakei* subsp. *sakei* CBA3635, *W. cibaria* CBA3636, and the reference strains (Fig. 4). The phylogenetic tree showed that the three strains are located within two large clades. In addition, *L. suantsaii* CBA3634 was closely related to the reference strain *Levilactobacillus suantsaii* L88T, and *L. sakei* subsp. *sakei* CBA3635 was closely related to the reference strains *Latilactobacillus sakei* subsp. *sakei* JCM 1157T and *Latilactobacillus sakei* subsp. *carnosus* DSM 15831T.

### Genomic sequence accession numbers

The genomes of *L. suantsaii* CBA3634, *L. sakei* subsp. *sakei* CBA3635, and *W. cibaria* CBA3636 were deposited in NCBI with the accession numbers CP059603–CP059608, CP059697–CP059698, and CP059699–CP059701, respectively.

### Discussion

Bacteria play a vital role in food fermentation. Bacterial community structure, particularly LAB community structure, is closely associated with the texture, flavor, nutrients and quality of fermented produce. However, the understanding of bacterial composition is still limited to several major species responsible for traditional fermented vegetables (Liu and Tong 2017). In this study, the bacterial diversities associated with six fermented vegetables from Zhejiang were investigated. HTS analysis identified 24 phyla, 274 families, and 569 genera based on the OTUs identified in the six samples. The major phyla were Firmicutes and Proteobacteria, the relative abundance of which ranged from 67.85 to 97.48%. This indicated a firm correlation between the two types of bacteria and vegetable fermentation. In fact, most of the previous reports have also found that fermented vegetables mainly contain Firmicutes and Proteobacteria at the phylum level (Liang et al. 2018; He et al. 2020).

At the genus level, however, the abundance of different genera varied greatly among the diverse fermented vegetables. This result was in accordance with the results of previous studies. For example, *Lactobacillus*-related genera and *Pediococcus* were found to be the major genera in the suancai samples (Liu et al. 2019a), whereas *Bacillus* and *Bacteroides* were the main genera in shuidouchi samples (Chen et al. 2019). The results of the present study showed that *Brevibacterium* was the most abundant genus in C1 sample. *Lactobacillus*-related genera and *Vibrio* were the most abundant genera in C2 sample and C6 sample. *Enterobacter* and *Cobetia* were the most abundant genera in C3 sample and C5 sample. *Chromohalobacter* was the most abundant in C4 sample. These differences in bacterial community may result from the different types of raw materials used during fermentation. In addition, besides the effects of the raw vegetable used, the effects of manufacturing processes or environmental conditions such as temperature, humidity and salinity are also significant on bacterial community and the final quality of the fermented vegetables (Xu et al. 2020).

Comparing the results obtained from the culture-dependent and culture-independent methods, many microorganisms such as *Lactobacillus*-related genera, *Leuconostoc*, and *Weissella* were encountered using both methods. In particular, *Lactobacillus*-related genera were found to be abundant by both approaches, which effectively confirmed the existence and composition of these microbes and community in the fermented vegetables. However, for the C4 sample, no colonies were obtained on the MRS medium. Some halophilic bacteria, such as *Chromohalobacter*, *Halomonas*, and *Halanaerobium*, were dominant in the C4 sample, and these bacteria need to be grown in a salty medium. Thus,
the development of a more specific medium for target strain growth and reproduction will be critical for future study.

Whole-genome analysis of strains CBA3634, CBA3635, and CBA3636 showed that these three LAB had a large number of genes associated with carbohydrate metabolism. Carbohydrate metabolism includes subcategories related to glucose, fructose, mannose, galactose, ascorbate, aldarate, starch, sucrose, amino sugar, nucleotide sugar, pyruvate, glyoxylate, dicarboxylate, and more. These results indicate that *L. suantsaii* CBA3634, *L. sakei* subsp. *sakei* CBA3635, and *W. cibaria* CBA3636 are capable of metabolizing various carbohydrates. In addition, a tertiary-level analysis of...
the carbohydrate metabolism in the genomes of the three strains indicated that their genes are involved in utilizing a variety of carbon sources such as starch, sucrose, pentose, galactose, fructose, and mannose. According to a previous report (Tashakor et al. 2017), L. sakei can indeed use different carbon sources, such as sucrose, maltose, fructose, and arabinose. Furthermore, it has been shown that Weissella can use glucose, D-maltose, D-ribose, mannitose, and trehalose. This ability to use multiple carbon sources could explain why L. sakei subsp. sakei CBA3635 and W. cibaria CBA3636 are able to grow in the fermented vegetable environment, for it may enhance their survival, competitiveness, and persistence.

Active or ambiguous prophages were detected in the L. suantsaii CBA3634, L. sakei subsp. sakei CBA3635, and W. cibaria CBA3636 genomes. In general, the presence of prophages in the genome has been considered to have a negative effect on the host, but recently it has been shown that prophages can provide several benefits to the host. Prophages can act in bacterial cellular processes, such as antibiotic resistance, stress response, and virulence, and also mediate defense against superinfection (Song et al. 2019). The prophages were found in the genomes of LAB including Lactobacillus, Weissella, and Leuconostoc. Prophages present in LAB genomes can help the host to survive in their habitats (Durmaz et al. 2008; Wang et al. 2010; Liu et al. 2009; Joseph et al. 2016; Panthee et al. 2019).

Some strains of L. sakei are known to produce bacteriocins, such as sakacin A, sakacin G, sakacin P, sakacin Q, and sakacin X (Kim et al. 2020). The bioinformatic analysis performed in the present study showed that the L. sakei subsp. sakei CBA3635 genome contained a sakacin P response regulator gene. However, the sakacin P structural gene sppA was not found in the genome. Mtr et al. also found some of L. sakei strains that did not produce sakacin P, but contained sakacin P-related synthetic genes (Mtr et al. 2005). However, the role of the sakacin P-related genes in non-producers is not known. These results indicate that L. sakei subsp. sakei CBA3635 cannot produce sakacin. In addition, Weissella species have been known to synthesize extracellular exopolysaccharides (EPS) (Ye et al. 2018). EPS-producing LAB have been known to able to tolerate gastrointestinal conditions, reduce pathogenic biofilm formation, and help in adhesion to epithelial cells than non-EPS-producing LAB. The bioinformatic analysis performed in the present study showed that W. cibaria CBA3636 genome contained the exopolysaccharide biosynthesis protein, which is an EPS-related gene. Nucleotide sugar biosynthesis is essential for EPS. Through further exploration of the KEGG database, it was found that W. cibaria CBA3636 had a high number of genes (29 genes) related to amino sugar and nucleotide sugar biosynthesis. This shows that W. cibaria CBA3636 is a potential EPS-producing bacterial strain.

Conclusion

Using HTS, the bacterial community of six fermented vegetables from Zhejiang Province in China was found to be highly diverse. Firmicutes and Proteobacteria were the main phyla found in all of the fermented vegetables. However, Brevisbacterium was the major genus in Xiaoshan pickled radish; Lactobacillus-related genera and Vibrio were dominant in fermented potherb mustard and its brine; Enterobacter and Cobetia were the major genera in fermented radish and its brine; and Chromohalobacter was the major genus in tuber mustard. These results indicated that different types of raw materials and manufacturing processes led to differences in the bacterial genus present. A culture-dependent method was used to isolate the cultivable LAB strains. In total, 44 isolates were successfully obtained and identified by 16S rRNA gene sequencing. Among them, 30 strains were LAB strains, accounting for more than 68% of all the isolates. Whole-genome analysis of L. suantsaii CBA3634, L. sakei subsp. sakei CBA3635, and W. cibaria CBA3636 showed that these three bacteria had a large number of genes associated with carbohydrate metabolism. This illustrated that L. suantsaii CBA3634, L. sakei subsp. sakei CBA3635, and W. cibaria CBA3636 can grow in fermented vegetable environments.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00203-021-02375-7.

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Author contributions DL and SWR designed experiments. JZ and HSS analyzed experimental results and wrote the manuscript. CZ, YBK, and SWR conducted the experiments.

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Availability of data and material All data during this study were included in this article.

Declarations

Conflict of interest The authors declared that they had no competing interests.

Conflict to participate All the authors have approved the manuscript.

Conflict for publication All the authors have approved the manuscript.
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