Suitability of various DNA extraction methods for a traditional Chinese paocai system

Nan Zhao, Jialiang Cai, Chuchu Zhang, Zhuang Guo, Wenwei Lu, Bo Yang, Feng-Wei Tian, Xiao-Ming Liu, Hao Zhang, and Wei Chen

ABSTRACT

Traditional paocai brine (PB), which is continuously propagated by back-slopping and contains various species of lactic acid bacteria (LAB), is critical for the flavor of paocai. Culture-independent approaches are commonly used to investigate the microbial communities of fermented food. To evaluate the influence of different DNA (DNA) extraction methods on estimates of bacterial community profiles from 4 PBs, the lysis efficiency, DNA yield, purity and denaturing gradient gel electrophoresis (DGGE) profiles of V3 region of a 16S ribosomal ribonucleic acid gene were acquired. The cell lysis pattern of SDS + beads and Lysing matrix E beads (methods 3 and 4) showed higher cell lysis efficiency than SDS and SDS + Lysozyme (methods 1 and 2) in all PBs. SDS + beads obtained the largest DNA yield of the 4 methods. Moreover, methods 3 and 4 resulted in higher H0 values and generated more global bacteria profiles than other methods. Overall, our results demonstrate that the properties of PB significantly affect the efficiency of DNA extraction methods. Methods 3 and 4 were both suitable for DNA extraction from PB. Method 3 is more economic, simple and rapid than method 4 for large-scale studies of the bacterial profiles of PB.

KEYWORDS

16S rDNA; diversity; DNA extraction; Fermented vegetables; Paocai

Introduction

Paocai, a style of Chinese traditional pickled vegetable with a long history that can be traced back to 1000 B.C., is commonly consumed in Asia. The traditional paocai fermentation method is still popularly used and the annual production of traditionally fermented paocai is estimated to be 180 million tons. Vegetables are pretreated and immersed in paocai brine (PB) and then kept at room temperature for 6–10 d in pickle jars. PB contains a stable lactic acid bacteria (LAB) community and large compounds that are obtained by continuously using the brine for a long time, even up to a century for some old brands, a maintenance process known as back-slopping. The quality of traditional paocai depends not only on the microbes that live on the vegetable, but also on the microflora in the PB. Although the microflora play a critical role in producing high-quality paocai, both on domestic and industrial scales, little attention has been paid to PB microflora.

A more comprehensive understanding of the microbial community in PB could help to improve the quality of paocai. Multiple species of bacteria have been isolated from PB using culture-dependent methods, but the global profile of microflora has received less attention in previous studies using culture-independent methods.

Culture-independent methods have been widely used to obtain global microbial community profiles of different ecological systems to overcome the limitations of culture-dependent methods. To obtain more global and reliable information about microbiota, the extraction of genomic DNA is a crucial step in culture-independent methods. Properties of the sample, such as the species of microbe present and interference in the sample, influence the efficiency of DNA extraction methods. For example, lysis methods can bias the content of the DNA extract toward specific groups of bacteria, especially some Gram-positive

CONTACT

Wei Chen  chenwei66@jiangnan.edu.cn  School of Food Science and Technology, Jiangnan University, Wuxi 214122, P.R. China.

© 2017 Taylor & Francis
bacteria. Moreover, interference from carbohydrates, proteins, polyphenols, and salts might protect microbes from lysis, or persist in the extract and act as polymerase chain reaction (PCR) inhibitors, thus affecting the representation of the microbial communities in the sample. In general, suitable DNA extraction methods are evaluated and developed for specific samples. Large compounds produced by microbes or released from vegetables accumulate in PB during back-slopping. An increase in compounds such as polysaccharides, polyphenols and proteins in PB over time could influence the efficiency of the DNA extraction method that is commonly used to obtain DNA from normal fermented vegetables. Thus, to obtain high-quality DNA from PB for further large-scale studies, the suitability of DNA extraction methods needs to be evaluated.

In this study, we present a comprehensive evaluation of a large-scale in situ DNA extraction method for PB with different back-slopping times (Table 2). Three laboratory-developed DNA extraction methods and one commercial kit were used. Each method was evaluated with respect to (1) cell lysis efficiency; (2) gDNA yield and purity; and (3) the reproducibility of the community profiles generated by DGGE of 16S rRNA genes. An efficient method was chosen to extract high-quality DNA to perform a reliable analysis of the microbial communities associated with PB.

### Materials and methods

#### Sample collection

Four samples of PB were collected from 3 provinces in China (Sichuan, Jiangsu, and Beijing) and were frozen immediately in liquid nitrogen. The samples were transferred to the laboratory within 24 h in an ice bucket and stored at $-80^\circ$C. The pH levels were then measured using a digital pH meter (Mettler Toledo 320-s, Shanghai, China). Total nitrogen and acid were determined according to the AOAC (1997) and protein content was determined by means of the biuret reaction with serum as standard.

#### DNA extraction

Three laboratory-developed DNA extraction methods and one commercial kit were used in our study (Table 1). Each method was based on the direct lysis of the cells in the sample, with subsequent recovery and purification of nucleic acids.

### Table 1. Comparison of DNA extraction methods and kits used in this study.

| Method | Cell lysis | Homogenization technique | Adsorption of PCR inhibitors | DNA capture technique | Approximate completion time (min) |
|--------|------------|---------------------------|-----------------------------|-----------------------|-------------------------------|
| Method 1 | SDS | Fast Prep instrument (speed 6.0, 40 s) | MT buffer and PPS solution | Isopropanol precipitation | 120 |
| Method 2 | SDS + lysozyme | Fast Prep instrument (speed 6.0, 40 s) | CI | Isopropanol precipitation | 120 |
| Method 3 | SDS + beads | Fast Prep instrument (speed 6.0, 40 s) | CI | Isopropanol precipitation | 30 |
| Method 4 | Lysing matrix E + beads | Fast Prep instrument (speed 6.0, 40 s) | MT buffer and PPS solution | Binding matrix suspension and SPIN^TM | 90 |

#### Table 2. Properties of PBs used for gDNA extraction.

| Sample | Usage time (months) | pH | Total nitrogen (mg/L) | Total acid (% lactic acid) | Polysaccharide (mg/ml) | Protein (mg BSA/ml) |
|--------|---------------------|----|-----------------------|---------------------------|-----------------------|---------------------|
| PB1    | 48                  | 3.63 ± 0.03^b† | 1.50 ± 0.06^d† | 1.22 ± 0.15 | 4.92 ± 0.03 | 1.74 ± 0.03^d† |
| PB2    | 36                  | 3.93 ± 0.02^c† | 1.44 ± 0.01 | 1.65 ± 0.03 | 5.64 ± 0.03 | 1.55 ± 0.02^c† |
| PB3    | 3                   | 3.02 ± 0.02^a† | 0.42 ± 0.01 | 0.67 ± 0.02 | 1.41 ± 0.15 | 0.32 ± 0.03^b† |
| PB4    | 2                   | 3.70 ± 0.01^b† | 0.33 ± 0.04 | 0.20 ± 0.01 | 1.17 ± 0.25 | 0.23 ± 0.02^a† |

Note: Each value represents the mean of 3 independent samples. The superscript letters a, b, c, and d indicate statistically significant differences at a p value <0.05 within each row comparison.
phosphate, 1.5 mol/L NaCl) and 5 μl of proteinase K (10 mg/ml) by shaking for 30 min at 37°C. The following procedures were same as the method of Lee et al.

**Method 2** (lysis by chemicals and enzymes), modified from Method 1.
We mixed 900 μl of DNA extraction buffer (100 mmol/L Tris–HCl pH 8.0, 100 mmol/L sodium EDTA, 100 mmol/L sodium phosphate, 1.5 mol/L NaCl), 5 μl of proteinase K (10 mg/ml), and 20 μl lysozyme buffer (20 mg/ml, Sigma-Aldrich) with the pellets described above and incubated them at 37°C for 30 min. After incubation, 100 μl of 20% (w/v) SDS was added, and the tubes were further incubated at 65°C for 1 h with gentle inversion every 15 to 20 min. The suspension was transferred to a microcentrifuge tube. The rest of the procedure was the same as for method 1.

**Method 3**
We mixed 1 mL SDS extraction buffer (100 mmol/L Tris–HCl pH 8.0, 100 mmol/L sodium EDTA, 100 mmol/L sodium phosphate, 1.5 M NaCl, 2% SDS) with the pellets described above. The suspension was agitated with a mixture of beads (0.10 g Zirconia/Silica beads (0.1 mm) + 0.20 g glass beads (1.0 mm) + 1 g glass beads (2.5 mm)) (Biospec Products, Bartlesville, OK, USA) in a Fast Prep® Instrument (Qbiogene Inc., USA) at speed 6 for 40 s. After centrifugation at 10,000 g for 5 min at room temperature, the supernatants were collected and transferred into 2 ml centrifuge tubes. The remaining parts of the procedure were the same as for method 1.

**Method 4** (FastDNA® SPIN Kit) (Qbiogene Inc., USA).
The genomic DNA was extracted according to the manufacturer’s instructions (see Table 1).

**Cell lysis efficiency**
The cell lysis efficiency was estimated according to the method of Zhou et al.22

**DNA yield and purity**
The gDNA yields and quality were assessed using a Quant-iTTM PicoGreen® dsDNA Kit (Molecular Probes, Inc., Eugene, OR) and measuring absorbance at 260 and 280 nm using a spectrophotometer (NanoDrop2000TM; Thermo Scientific Inc., West Palm Beach, FL). The DNA extractions were performed in triplicate for each DNA extraction method.

**Microbial diversity**
The composition of the microbial community was evaluated by performing a DGGE based on the DNA (16S rRNA gene). The microbial profiles yielded by the DGGE were used as a proxy to evaluate the diversity of the microbial community retrieved from the PBs by the DNA extraction methods described above. PCR amplification and DGGE was estimated according to the method of Ai et al.23

**Microbial diversity analysis**
We used the Rolling disk method with Quantity One software (Bio-Rad laboratories Inc.), which normalizes the band pattern from electrophoresis for identification of each band. The DGGE fingerprints were interpreted in terms of their band richness (number of predominant DGGE bands/population). The pixel intensity of each band was detected by Quantity One software and is expressed as relative abundance (Pᵢ). Shannon’s index of diversity (H’) was determined as

\[ H' = -\sum Pᵢ\ln Pᵢ \]

where \( Pᵢ \) is often the proportion of individuals who belong to each species in the data set of interest. The statistical significance of the bacterial structure for the different extraction methods was assessed by multivariate analysis of variance using the PCA scores in MATLAB 2010b (The MathWorks, Inc., USA).

**Statistical analyses**
All analyses were conducted in triplicate. The statistical analyses were performed using Matlab® and SPSS 11.0. Duncan’s multiple range test was used for comparison of group means with significant differences defined at \( p < 0.05 \).

**Results**

**Properties of PB**
The pH of the PBs ranged from 3.02 to 3.93 (Table 2). The total nitrogen content (0.33 to 1.50 mg/L) and protein content (0.23 to 1.74 mg bovine serum albumin per mL) increased with usage time. PB-2 had the highest total acid and polysaccharide content. The results suggest that the properties of the various PBs were significantly different. These different properties may influence how suitable the DNA extraction
method developed for normal fermented vegetables is for extracting DNA from PB. A more appropriate DNA extraction method needs to be chosen for the various PB samples.

**Efficiency of DNA extraction methods for different PB properties**

In preliminary experiment, the efficiency of the popular DNA extraction kits including QIAamp® DNA Stool Mini Kit, the E.Z.N.A.® Soil DNA Kit, and FastDNA® SPIN Kit (method 4) was investigated, and FastDNA® SPIN Kit (method 4), the widely used in DNA extraction from kimchi (a fermented vegetable from Korean), presented the most efficient performance among these commercial kits. Hence, method 4 was used as a positive control in the present study.

**Cell lysis efficiency**

Four cell lysis methods were used to lyse the microbes in the PB samples. As shown in Fig. 1, methods 1 (SDS) and 2 (SDS + lysozyme) had significantly lower cell lysis efficiency than methods 3 (SDS + beads) and 4 (MP beads) in all PB samples, especially those with longer usage time. DNA extraction methods associated with agitation with beads (methods 3 and 4) did not have significantly different cell lysis efficiencies across the 4 samples. Overall, cell lysis methods that agitate with beads were more suitable and able to break microbial cells in PBs than were other methods.

**Yield and purity of DNA**

The yield and purity of DNA are important criteria to evaluate the efficiency of DNA extraction methods. Fig. 2(A) clearly shows that the DNA samples extracted by method 3 obtained the highest yield from 4 PB samples (3.52 to 42.17 μg/ml). The lowest yields were determined in the DNA samples extracted by method 2 (0.70 to 20.29 μg/mL). Although there is no significant difference between the lysis efficiency of methods 3 and 4, a lower DNA yield was obtained by method 4 than by method 3. This might be due to the stricter purification methods of method 4. The ratio of OD_{260} to OD_{280} was used to evaluate the purity of the

![Figure 1. Efficiency of cell lysis with different DNA extraction methods. Note: Values presented are means (mean ± SD) of 3 independent replicates. Different letters (a, b, c, d) represent significant differences (p < 0.05). DNA extraction methods: Method 1, Method 2, Method 3, and Method 4.](image)

![Figure 2. DNA yields and purity from 4 PBs using 4 DNA extraction methods. Note: (A) DNA quantified with PicoGreen dsDNA Kit. (B) DNA qualified by OD_{260}/OD_{280} with NanoDrop. Values presented are means (mean ± SD) of 3 independent replicates. The horizontal line shows the ratio at 1.8, which is the index of optimal DNA purity. Significant differences (p < 0.05) between the strains are indicated with different letters (a, b, c, d) above the bars. DNA extraction methods: Method 1, Method 2, Method 3, and Method 4.](image)
The ratio of OD$_{260}$ to OD$_{280}$ in the DNA extracts obtained by methods 3 and 4 was greater than 1.8, and that of the extracts from PB1 obtained by methods 1 and 2 was less than 1.8. This may indirectly demonstrate the existence of proteins in the DNA extracts yielded by methods 1 and 2, which might influence the later DNA amplification, but not in the extracts yielded by methods 3 and 4. In general, methods 3 and 4 were more applicable for DNA extraction from the various PB samples.

**Representing richness and diversity**

To reveal the effects of the DNA extraction methods on the structure of the bacterial community, DGGE technology was used to describe the bacterial community of PB-2, which had more complex interference content than other samples (Table 2). As shown in Fig. 3, the number and intensity of the bands retrieved from methods 3 and 4 were significantly greater than those obtained from methods 1 and 2. The band-type index is commonly used to characterize the mean community richness in a community. Methods 3 and 4 had more band numbers (both 19 ± 0) than methods 1 (6.67 ± 2.08) and 2 (13.33 ± 1.16). The α-diversities, as estimated by the Shannon diversity index, showed the same tendency (Fig. 4). It is worth mentioning that there were no significant differences in these 2 indices between the DNA samples extracted by methods 3 and 4 ($p > 0.05$, Fig. 4). A Venn diagram was constructed to pinpoint the levels of similarity (overlap) between these methods. According to the diagram (Fig. 5), the bands of the DNA samples extracted by methods 3 and 4 covered almost all of the bands from the other methods. These results indicate that methods 3 and 4 obtained more global bacteria profiles from PB-2 than did the other DNA extraction methods. Multivariate analysis of variance also revealed no significant differences between the microbial

![Figure 3](image-url)  
*Figure 3.* DGGE gel illustrating reproducibility of 4 DNA extraction methods based on DGGE profiles from PB-2. Note: Lanes 1–3, 4–6, 7–9, and 10–12 show DGGE profiles generated from 3 separate extractions based on M1, M2, M3, and M4, respectively.

![Figure 4](image-url)  
*Figure 4.* Band-type richness (A) and Shannon’s index of diversity ($H^*$) (B) calculated from DGGE community profiles of bacteria detected on PB-2 using 4 DNA extraction methods. Values presented are means (mean ± SD) of 3 independent replicates. Different letters represent significant differences ($p < 0.05$). DNA extraction methods: (■) Method 1, (□) Method 2, (■) Method 3, and (□) Method 4.
communities retrieved using methods 3 and 4 (p = 0.423), but they were significantly different from those retrieved by methods 1 (p = 2.960e-06) and 2 (p = 5.026e-14) (Fig. 6).

The bacterial community of the other 3 samples was then evaluated by DGGE. As shown in Fig. 7, PCR products were not successfully obtained from the DNA obtained from PB-1 using methods 1 and 2, suggesting that the properties of PB significantly affect the efficiency of DNA extraction in methods 1 and 2. The community profiles retrieved using methods 3 and 4 showed more global profiles than those from methods 1 and 2 in all samples.

All of the results demonstrate that DNA extraction methods can influence the information obtained about the bacterial communities of PB, especially in longer usage time PB. Methods 3 and 4 were better able to reflect the bacterial communities regardless of the properties of PB.

**Discussion**

Different cell lysis methods have biases toward certain strains, such that an accurate representation of the microbial community can be achieved.\textsuperscript{12,32} For example, Gram-negative cells tend to be lysed more easily than Gram-positive cells. Thus, gentler cell lysis methods may not sufficiently break down Gram-positive cells, which could enrich extracts with DNA from Gram-negative bacteria. For fermented vegetables that contain abundant Gram-positive organisms (e.g., lactic acid bacteria), relatively stronger lysis methods (methods 3 and 4) could help to obtain more comprehensive bacterial information. Moreover, previous studies have found that a large amount of other substances in the matrix affects the efficiency of cell lysis.\textsuperscript{8} The substrates produced by microbes and/or raw...
material residues such as polysaccharides, organic acids, and polyphenols increase with fermentation time.\textsuperscript{33} In our study, the polysaccharide level was about 5 times higher in PB-1 and PB-2 than in PB-3 and PB-4. SDS has been the most widely used cell lysis treatment of DNA extraction from pure cultures or normal fermented vegetables,\textsuperscript{22} but it was inefficient in PB-1 and PB-2. It might be that older PBs accumulate more interference compounds, such as proteins and polysaccharides, during back-slopping. Polysaccharides are innate protectors of bacterial cells.\textsuperscript{34} The cells may cluster together and are enclosed by polysaccharides, which may protect them from chemical reagents, including SDS and enzymes.\textsuperscript{35} Thus, the lysis in methods 1 and 2 was too mild to effectively lyse the cells. Agitation with beads (methods 3 and 4) may disperse the bacterial cell clusters and enable efficient lysis.

Another factor affecting an extraction method’s DNA quality is the subsequent loss during purification.\textsuperscript{8} The choice of an extraction method often involves a trade-off between the cost (materials and labor), the optimal yield of DNA and the removal of substances that could influence the PCR reaction.\textsuperscript{36} The results of our research indicate that the DNA extracted by method 3 had the highest yield, but it was not as pure as the DNA from method 4. However, it is worth mentioning that amplification products were successfully obtained by both methods 3 and 4. Overall, methods 3 and 4 are both suitable for DNA extraction from various PB samples.

Previously, methods of extracting DNA from food have focused on the detection of particular microbes, including spoilage bacteria,\textsuperscript{37,38} pathogenic bacteria,\textsuperscript{39,40} and some bacteria dominant in fermented foods.\textsuperscript{23} DNA yield and purity and PCR amplification products are usually used to evaluate the efficiency of DNA extraction methods for detection of a certain bacteria in the food matrix.\textsuperscript{41} However, to investigate the microbial communities in fermented foods, DNA extraction methods should be suitable for a wide range of microorganisms. Microbial profiles might be important for the evaluation of DNA extraction methods for microbial community analysis. In our study, DNA extraction methods significantly affected the $H^\prime$ of the bacterial communities retrieved from PB-2, as also recorded for other environmental samples.\textsuperscript{7,10,12,42} Although previous studies considered method 1 to be an efficient DNA extraction method for fermented vegetables\textsuperscript{21} our results indicate that the bacterial community profiles generated from this method had significantly lower $H^\prime$ values than did those from methods that included agitation with beads. This might be due to the lower lysis efficiency of these DNA extraction methods. Methods 3 and 4 obtained more global DGGE profiles than did method 1 or 2, and similar bacteria profiles for all 4 PB samples. According to a comprehensive evaluation of DNA extraction methods, methods 3 and 4 were recognized as suitable for PB bacterial profile analysis. It is worth noting that method 3 is more economic, simple and quick than method 4, and therefore it might be more applicable to large-scale studies.

Our results demonstrate that the properties of PB affect the DNA extraction efficiency of methods 1 and 2. Methods 3 and 4 are both suitable for use in DNA extraction for bacterial profile analysis of PB samples with various properties. Moreover, method 3 proved to be more simple and rapid than other methods for large-scale studies of the bacterial profiles of PB.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

**Funding**

This work was supported by the National Basic Research Program of China (973 Program No. 2012CB720802), the Program for Changjiang Scholars and Innovative Research Team in University (IRT1249), the 111 Project B07019, and Program of Collaborative Innovation Center of Food Safety and Quality Control in Jiangsu Province.

**References**

[1] Xiong T, Peng F, Liu YY, Deng YJ, Wang XY, Xie MY. Fermentation of Chinese sauerkraut in pure culture and binary co-culture with Leuconostoc mesenteroides and Lactobacillus plantarum. LWT-Food Science and Technology 2014; 59(2):713-7; PMID:ISI:000342527400017; https://doi.org/10.1016/j.lwt.2014.05.059

[2] Jung JY, Lee SH, Kim JM, Park MS, Bae JW, Hahn Y, Madsen EL, Jeon CO. Metagenomic analysis of kimchi, a traditional Korean fermented food. Appl Environ Microb 2011; 77(7):2264-74; PMID:21317261; https://doi.org/10.1128/AEM.02157-10

[3] Xiong T, Guan QQ, Song SH, Hao MY, Xie MY. Dynamic changes of lactic acid bacteria flora during Chinese sauerkraut fermentation. Food Control 2012; 26 (1):178-81; PMID:ISI:000301997000030; https://doi.org/10.1016/j.foodcont.2012.01.027
[4] Jung JY, Lee SH, Jeon CO. Kimchi microflora: history, current status, and perspectives for industrial kimchi production. Appl Microbiol Biot 2014; 98(6):2385-93; PMID:24419800; https://doi.org/10.1007/s00253-014-5513-1

[5] Wolfe BE, Button JE, Santarelli M, Dutton RJ. Cheese rind communities provide tractable systems for in situ and in vitro studies of microbial diversity. Cell 2014;158 (2):422-33; PMID:25036636; https://doi.org/10.1016/j.cell.2014.05.041

[6] Ercolini D. High-throughput sequencing and metagenomics: moving forward in the culture-independent analysis of food microbial ecology. Appl Environ Microb 2013; 79(10):3148-3155; PMID:23475615; https://doi.org/10.1128/AEM.00256-13

[7] Yuan S, Cohen DB, Ravel J, Abdó Z, Forney LJ. Evaluation of methods for the extraction and purification of DNA from the human microbiome. Plos One 2012; 7(3): e33865-74; PMID:22457796; https://doi.org/10.1371/journal.pone.0033865

[8] Guo F, Zhang T. Bias during DNA extraction of activated sludge samples revealed by high throughput sequencing. Appl Microbiol Biot 2013; 97(10):4607-16; PMID:22760785; https://doi.org/10.1007/s00253-012-4244-4

[9] Hazen TC, Rocha AM, Tchetchman SM. Advances in monitoring environmental microbes. Curr Opin Biotech 2013; 24(3):526-33; PMID:23183250; https://doi.org/10.1016/j.copbio.2012.10.020

[10] Rocha J, Coelho FJ, Peixe L, Gomes NC, Calado R. Optimization of preservation and processing of sea anemones for microbial community analysis using molecular tools. Sci. Rep. 2014; 4:6986-90; PMID:25384534; https://doi.org/10.1038/srep06986

[11] Vishnivetskaya TA, Layton AC, Lau MC, Chauhan A, Cheng KR, Meyers AJ, Murphy JR, Rogers AW, Saarunya GS, Williams DE, et al. Commercial DNA extraction kits impact observed microbial community composition in permafrost samples. FEMS Microbiol. Eco. 2014; 87 (1):217-30; PMID:24102625; https://doi.org/10.1111/1574-6941.12219

[12] Carrigg C, Rice O, Kavanagh S, Collins G, O’Flaherty V. DNA extraction method affects microbial community profiles from soils and sediment. Appl Microbiol Biot 2007; 77(4):955-64; PMID:17960375; https://doi.org/10.1007/s00253-007-1219-y

[13] Sagar K, Singh SP, Goutam KK, Konwar BK. Assessment of five soil DNA extraction methods and a rapid laboratory-developed method for quality soil DNA extraction for 16S rDNA-based amplification and library construction. J. Microbiol. Methods 2014; 97:68-73; PMID:24280193; https://doi.org/10.1016/j.jmimet.2013.11.008

[14] Inceoglu O, Hoogwout EF, Hill P, van Elsas JD. Effect of DNA Extraction Method on the Apparent Microbial Diversity of Soil. Appl Environ Microb 2010; 76 (10):3378-82; PMID:000277388200037; https://doi.org/10.1128/AEM.02715-09

[15] Wu GD, Lewis JD, Hoffmann C, Chen YY, Knight R, Bittinger K, Wang J, Chen J, Berkowsky R, Nessel L, et al. Sampling and pyrosequencing methods for characterizing bacterial communities in the human gut using 16S sequence tags. BMC Microbiol 2010; 10:206-20; PMID:200281802500002; PMID:20673359; https://doi.org/10.1186/1471-2180-10-206

[16] Peng X, Yu KQ, Deng GH, Jiang YX, Wang Y, Zhang GX, Zhou HW. Comparison of direct boiling method with commercial kits for extracting fecal microbiome DNA by Illumina sequencing of 16S rRNA tags. J. Microbiol. Methods 2013; 95(3):455-62; PMID:ISI:000329003500024; PMID:23899773; https://doi.org/10.1016/j.mimet.2013.07.015

[17] Liao J, Liu YF, Ku T, Liu MH, Huang Y. Qualitative and quantitative adulteration identification of milk powder using the DNA with novel extraction method. J. Dairy Sci. 2017; 100(3):1657-63; PMID: 28088415; https://doi.org/10.3168/jds.2016-11990

[18] Yang X, Li H, Cheng T, Xia W, Lai YT, Sun H. Nickel translocation between metallochaperones HypA and UreE in Helicobacter pylori. Metalloids : integrated bi-metal science 2014; 6(9):1731-6; PMID:25010720; https://doi.org/10.1039/c4mt00134f.

[19] Tomita Y, Watanabe T, Takeuchi T, Nanbu A, Shinozaki N, Ikemi T, Fukushima K. Effects of surfactants on glucosyltransferase production and in vitro sucrose-dependent colonization by Streptococcus mutans. Archives of Oral Biology 1998; 43(9):735-40; PMID:ISI:000075994700009; PMID:9783828; https://doi.org/10.1016/S0003-9969(98)00065-X

[20] Gornall AG, Bardawill CJ, David MM. Determination of serum proteins by means of the biuret reaction. J Biol Chem 1949; 177(2):751-66; PMID:18110453

[21] Lee JS, Heo GY, Lee JW, Oh YJ, Park JA, Park YH, Pyun YR, Ahn JS. Analysis of kimchi microflora using denaturing gradient gel electrophoresis. Int J Food Microbiol 2005; 102(2):143-150; PMID:15992614; https://doi.org/10.1016/j.ijfoodmicro.2004.12.010

[22] Zhou JZ, Bruns MA, Tiedje JM. DNA recovery from soils of diverse composition. Appl Environ Microb 1996; 62 (2):316-22; PMID:A1996TT69000002

[23] Ai CQ, Zhang QX, Ren CC, Wang G, Liu XM, Tian FW, Zhao JX, Zhang H, Chen YQ, Chen W. Protective effect of Streptococcus thermophilus CCFM218 against house dust mite allergy in a mouse model. Food Control 2015; 50: 283-290; PMID:WOS:000347581100039; https://doi.org/10.1016/j.foodcont.2014.08.040

[24] Ono H, Nishio S, Tsurii J, Kawamoto T, Sonomoto K, Nakayama J. Monitoring of the microbiota profile in nukadoko, a naturally fermented rice bran bed for pickling vegetables. J Biosci Bioeng 2014; 118(5):520-5; PMID:WOS:000347141300006; PMID:24906947; https://doi.org/10.1016/j.jbiosc.2014.04.017

[25] Jøsøf sen MH, Andersen SC, Christensen J, Hoofar J. Microbial food safety: Potential of DNA extraction methods for use in diagnostic metagenomics. Journal of...
[26] Yang L, Yang HL, Tu ZC, Wang XL. High-Throughput Sequencing of Microbial Community Diversity and Dynamics during Douchi Fermentation. Plos one 2016; 11(12):e0168166; PMID:27992473; https://doi.org/10.1371/journal.pone.0168166

[27] Zhang X, Zhao J, Du X. Barcoded pyrosequencing analysis of the bacterial community of Daqu for light-flavour Chinese liquor. Lett Appl Microbiol 2014; 58(6):549-55; PMID:24471485; https://doi.org/10.1111/lam.12225

[28] Liu SP, Yu JX, Wei XL, Ji ZW, Zhou ZL, Meng XY, Mao J. Sequencing-based screening of functional microorganism to decrease the formation of biogenic amines in Chinese rice wine. Food Control 2016; 64:98-104; PMID:WOS:000371189000015; https://doi.org/10.1016/j.foodcont.2015.12.013

[29] Jeong SH, Jung JY, Lee SH, Jin HM, Jeon CO. Microbial succession and metabolite changes during fermentation of dongchimi, traditional Korean watery kimchi. Int J Food Microbiol 2013; 164(1):46-53; PMID:23587713; https://doi.org/10.1016/j.ijfoodmicro.2013.03.016

[30] Jeong SH, Lee SH, Jung JY, Choi EJ, Jeon CO. Microbial succession and metabolite changes during long-term storage of Kimchi. J Food Sci 2013; 78(5):M763-9; PMID:23550842; https://doi.org/10.1111/j.1750-3841.12095

[31] Jeong SH, Lee HJ, Jung JY, Lee SH, Seo HY, Park WS, Jeon CO. Effects of red pepper powder on microbial communities and metabolites during kimchi fermentation. Int J Food Microbiol 2013; 160(3):252-9; PMID:23290232; https://doi.org/10.1016/j.ijfoodmicro.2012.10.015

[32] de Lipthay JR, Enzinger C, Johnsen K, Aamand J, Sorensen SJ. Impact of DNA extraction method on bacterial community composition measured by denaturing gradient gel electrophoresis. Soil. Biol. Biochem 2004;36(10):1607-14; PMID:000224524600011; https://doi.org/10.1016/j.soilbio.2004.03.011

[33] Mamlouk D, Hidalgo C, Torija MJ, Gullo M. Evaluation and optimisation of bacterial genomic DNA extraction for no-culture techniques applied to vinegars. Food Microbiol 2011; 28(7):1374-1379; PMID:21839388; https://doi.org/10.1016/j.fm.2011.06.009

[34] Flemming HC, Neu TR, D.J. W. The EPS matrix: the “house of biofilm cells.” J. Bacteriol 2007;189:7945-7; PMID:17675377; https://doi.org/10.1128/JB.00858-07

[35] Davies DG, Parsek MR, Pearson JP, Iglewski BH, Costerton JW, Greenberg EP. The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science 1998; 280(5361):295-8; PMID:000073082400054; PMID:9535661; https://doi.org/10.1126/science.280.5361.295

[36] Cankar K, Stebih D, Dre D, Zel J, Gruden K. Critical points of DNA quantification by real-time PCR-effects of DNA extraction method and sample matrix on quantification of genetically modified organisms. BMC Biotechnol 2006; 6:37-51; PMID:16907967; https://doi.org/10.1186/1472-6750-6-37

[37] Wielinga PR, de Heer L, de Groot A, Hamidjaja RA, Bruggeman G, Jordan K, van Rotterdam BJ. Evaluation of DNA extraction methods for Bacillus anthracis spores spiked to food and feed matrices at biosafety level 3 conditions. Int. J. Food Microbiol 2011;150(2-3):122-7; PMID:000295747900005; PMID:21864928; https://doi.org/10.1016/j.ijfoodmicro.2011.07.023

[38] Hosseini H, Hippe B, Denner E, Kollegger E, Haslerberger A. Isolation, identification and monitoring of contaminant bacteria in Iranian Kefir type drink by 16S rDNA sequencing. Food Control 2012; 25(2):784-8; PMID:000300740000054; https://doi.org/10.1016/j.foodcont.2011.12.017

[39] Dobhal S, Zhang G, Rohla C, Smith MW, Ma LM. A simple, rapid, cost-effective and sensitive method for detection of Salmonella in environmental and pecan samples. J. Appl. Microbiol. 2014; 117(4):1181-90; PMID:24947913; https://doi.org/10.1111/jam.12583

[40] Martinon A, Cronin UP, Quealy J, Stapleton A, Wilkinson MG. Swab sample preparation and viable real-time PCR methodologies for the recovery of Escherichia coli, Staphylococcus aureus or Listeria monocytogenes from artificially contaminated food processing surfaces. Food Control 2012;24(1-2):86-94; PMID:000297659100013; https://doi.org/10.1016/j.foodcont.2011.09.007

[41] Pirondini A, Bonas U, Maestri E, Visioli G, Marmiroli M, Marmiroli N. Yield and amplifiability of different DNA extraction procedures for traceability in the dairy food chain. Food Control 2010; 21(5):663-8; PMID:000274843400013; https://doi.org/10.1016/j.foodcont.2009.10.004

[42] Hwang C, Ling F, Andersen GL, LeChevallier MW, Liu WT. Evaluation of methods for the extraction of DNA from drinking water distribution system biofilms. Microbes Environ 2012; 27(1):9-18; PMID:22075624; https://doi.org/10.1264/jsme2.ME11132