

Diversity and community analysis of fermenting bacteria isolated from eight major Korean fermented foods using arbitrary-primed PCR and 16S rRNA gene sequencing

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Abstract Korean fermented foods are known to be beneficial for human health. Bacterial community studies have been conducted to figure out what roles the bacteria used to ferment these foods play in food fermentation. The metagenomic approach identifies both culturable and unculturable bacterial compositions, but this technique is limited in its ability to accurately determine the bacterial species from short 16S rRNA PCR products. In this study, we revisited the culture-dependent method using a relatively large number of bacterial isolates in an attempt to overcome the problem of bacterial identification, accepting that the unculturable bacterial population in each fermented food would be undetectable. Eight Korean fermented foods including kimchi, jeotgal, and meju were collected, and 1589 fermenting bacterial strains were randomly isolated. Bacteria were grouped by banding pattern using arbitrary-primed (AP) PCR prior to bacterial identification and sorted into 219 groups; 351 strains were not grouped because there was no identical AP-PCR band pattern. 16S rRNA sequence analysis identified the bacterial compositions of the fermented foods. As dominant genera, *Lactobacillus* and *Leuconostoc* strains were detected in four kimchi samples, *Staphylococcus* in three jeotgal samples, and *Enterococcus* and *Bacillus* in the meju sample. Interestingly, *S. Equorum* was most dominant in saeu-jeotgal, indicating that it is halophilic and may enhance the

fermentation flavor. Further comparative analysis of this study with previous metagenomic results revealed that bacterial communities in fermented foods are highly similar at the genus level but often differ at the species level. This bacterial community study is useful for understanding the roles and functional properties of fermenting bacteria in the fermentation process of Korean fermented foods.

Keywords Korean fermented foods · Fermenting bacteria · Arbitrary-primed PCR · Community analysis · Microbial diversity

Introduction

Food fermentation was originally performed to preserve traditional-style foods using naturally fermenting bacteria (Sieuwerts et al. 2008). It is recognized as a method to improve flavors and tastes and to promote human health (Sieuwerts et al. 2008). Korean traditionally fermented foods have been consumed in South Korea as every-day side dishes. They have attracted public interest for their potential health-promoting effects including lowering of blood pressure and serum cholesterol (Kim 2004; Kim et al. 2004), enhancement of immune function (Kim et al. 1997; Chae et al. 1998), promotion of calcium absorption (Park et al. 2014), as well as their anti-oxidative (Ryu et al. 1997, 2004a; Ryu et al. 2004b), anti-microbial (Sheo and Seo 2003; Lim and Im 2009), lipid-lowering (Kwon et al. 1999), and even anti-mutagenic activities (Shin et al. 1998; Son et al. 1998; Hur et al. 2000; Park et al. 2014). These host-beneficial effects have been suggested to originate from bioactive compounds in bacterial fermentation byproducts such as vitamins (Ku and Choi 1990; Cheigh

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1997), minerals (Cheigh et al. 1994), dietary fiber (Hur et al. 2000), and phytochemicals (Park et al. 2005). Although Korean fermented foods are high in sodium chloride and have uncontrolled concentrations and types of fermenting bacteria (Kim et al. 1995; Park et al. 2014), they have been generally accepted as healthy (Park et al. 2014). In addition to their flavor-enhancing qualities, the fermenting bacteria in these foods are thought to help in food preservation (Cheigh et al. 1994). Recently, selected fermenting bacteria were reported to produce industrially important compounds including fibrinolytic enzymes such as nattokinase for alleviation of thrombosis (Kim et al. 1996; Choi et al. 2009) and novel bacteriocins as natural food preservatives (Kim et al. 2011a), indicating that fermenting bacteria may be beneficial for host functions of Korean fermented foods (Choi et al. 2000; Ko and Ahn 2000; Lee and Paik 2001).

Although the health-promoting effects of Korean fermented foods have been studied, the role and composition of the complex fermenting bacteria in them are not yet fully understood, probably due to their complex bacterial composition via natural fermentation. Community bacterial analyses of Korean fermented foods including kimchi, meju, and jeotgal have been conducted using culture-dependent methods with bacterial culture media and culture-independent methods with bacterial DNA-based molecular techniques including metagenomics.

Comparison of the two methods for bacterial community analysis showed that even though major bacterial communities are highly similar at the genus level, their species are often different (Lee et al. 2010; Guan et al. 2011; Jung et al. 2011; Kim et al. 2011c; Jung et al. 2013; Jeong et al. 2014). A recent culture-dependent study on the kimchi bacterial community revealed the top five major fermenting bacteria: *Leuconostoc* (*Leuc*) *mesenteroides*, *L. citreum*, *Lactococcus* (*L*) *lactis*, *Weissella* (*W*) *koreensis*, *Bacillus* (*B*) *subtilis*, and *Lactobacillus* (*L*) *sakei* (Lee et al. 2010). However, metagenomic analysis of kimchi showed that the top five major fermenting bacteria were *L. mesenteroides*, *L. citreum*, *L. sakei*, *L. casei*, *W. paramesenteroides*, and *L. lactis* (Jung et al. 2011), indicating that the major bacteria of the bacterial community in kimchi are almost similar but slightly different at the *Weissella* species level between the two community analysis methods. In addition, the composition of fermenting bacteria in meju was also slightly different by two different community analysis methods. The culture-dependent method showed that *B. methylothrophicus*, *B. siamensis*, *Enterococcus* (*E*) *faecalis*, and *Staphylococcus* (*S*) *saprophyticus* are dominant in meju (Jeong et al. 2014). However, metagenomic analysis result revealed that *B. siamensis*, *B. amyloliquefaciens*, *B. atrophaeus*, *B. mojavensis*, and *E. durans* are the major fermenting bacteria (Kim et al. 2011c). Interestingly,

profiles of the bacterial communities in meju between the two different analysis methods are highly similar at the genus level but are often different at the species level, supporting the comparison of kimchi bacterial communities.

The culture-dependent method has a low detection limit, thus cannot detect all fermenting bacteria (Giraffa 2004). In addition, metagenomic analyses of bacterial communities generally use 16S rRNA universal primer sets synthesizing relatively short PCR products, such as 8F/338R, 784F/1061R, and 967F/1046R (Nikolaki and Tsiamis 2013). These short 16S rRNA PCR products often led to low resolution and identification accuracy in the bacterial community analysis (Ku and Lee 2014). Although the culture-dependent method cannot detect all bacteria in the community, bacterial identification using the culture-dependent method is more accurate. However, although the culture-independent method can detect both culturable and non-culturable bacteria in the community, the bacterial identification accuracy is relatively low, especially at the species level, thus explaining why there is not a perfect match between bacterial species found in the culture-dependent and culture-independent methods.

To overcome the low accuracy of bacterial identification in this study, the number of randomly picked fermenting bacteria was increased to approximately 200 colonies per fermented food sample. To investigate the bacterial communities in various Korean fermented foods, eight different food samples were collected, and 1589 fermenting bacteria were isolated from the collected samples. To efficiently identify the bacteria, isolated bacteria were grouped using the arbitrary-primed (AP) PCR method (Welsh and McClelland 1990, 1991; Cusick and O'Sullivan 2000), and each group was identified using 16S rRNA sequencing with long-range 16S rRNA universal primer sets making 580- or 890-bp 16S rRNA PCR products (Lane et al. 1985; Ku and Lee 2014). This study is useful to extend our knowledge about the composition and involvement of bacterial communities and food fermentation in Korean fermented foods. Furthermore, our results provide useful information for functional food applications of selected isolated fermenting bacteria via the culture-dependent method.

Materials and methods

Samples of Korean fermented foods

In this study, a total of eight different Korean fermented foods were collected: four kinds of kimchi (baek-kimchi, kkakdugi, pogi-kimchi, and mugeungi), three kinds of jeotgal (saeu-jeotgal, jogae-jeotgal, and myeolchi-jeotgal), and meju. Each fermented food was fermented in the

traditional Korean manner and purchased as a fermented commercial product. Kimchi samples were purchased from Daedo kimchi (Kwangju, South Korea), jeotgal from Gyeonggi seafood market (Inchon, South Korea), and meju from Jaekyung-doengjang (Paju, South Korea). After purchasing, all collected fermented foods were stored in a 4 °C refrigerator and used for isolation of fermenting bacteria within 1 week.

Isolation of fermenting bacteria and culture conditions

Each collected sample was thoroughly suspended in 0.1 % sterilized peptone water and serially tenfold diluted in the same buffer. Each diluted solution was plated on four different agar culture plates (below) and incubated aerobically or anaerobically at 37 °C for 3 days. After incubation, colonies were randomly picked and inoculated into the same kind of fresh broth media. The broth media were incubated at 37 °C for 1 day. The four different kinds of culture media used were Luria–Bertani (Difco, Detroit, MA, USA), lactobacilli de Man–Rogosa–Sharpe (Difco) supplemented with 0.05 % L-cysteine (final concentration; Sigma, St. Louis, MO, USA), M17 (Difco) supplemented with 0.5 % D-glucose (final concentration; Sigma), and Nutrient broth (Difco). All agar plates contained 1.8 % Bacto agar (Difco).

Arbitrary-primed (AP) PCR

For grouping of the isolated fermenting bacteria, the AP-PCR method was used based on the protocol of Cusick and O'Sullivan (2000) with the modification of annealing temperature of a single AP-PCR primer to 40 °C. PCR template DNAs were prepared from bacterial cells in broth media using Chelex 100 (Bio-Rad, Hercules, CA, USA), according to the protocol developed by Walsh et al. (1991). Bacterial groups were determined by comparing the PCR band patterns from AP-PCR.

16S rRNA gene sequencing

16S rRNA gene sequencing was conducted for identification of fermenting bacteria and further phylogenetic analysis. Total bacterial DNAs were extracted using Chelex 100 (Bio-Rad), and 16S rRNA genes were PCR-amplified using two long-range 16S rRNA universal primer sets for 580- and 890-bp PCR products (Lane et al. 1985, Ku and Lee 2014). The PCR reaction mixture contained 25 µl of final solution including 1 µl of DNA template, 2.5 µl 10× *Taq* DNA polymerase buffer (New England Biolabs (NEB), Ipswich, MA, USA), 0.2 mM of dNTP mix (Promega, Madison, WI, USA), 0.6 µM of each primer (926F/1505R or 16S-F/16S-R forward and reverse primers), and

2.5 U of *Taq* DNA polymerase (NEB). The PCR conditions were as follows: initial denaturation at 95 °C for 5 min followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 30 s, with 1 cycle of final elongation at 72 °C for 10 min. The 16S rRNA PCR products were purified using an AxyPrep™ DNA Gel Extraction Kit (Axygen, Tewksbury, MA). Sequencing of the 16S rRNA PCR products was conducted using 454 GS FLX+ system (Roche) and one of the 16S rRNA universal primers (forward or reverse primer) by Macrogen, Seoul, Republic of Korea.

Bioinformatics

Sequences of 16S rRNA PCR products were analyzed using the NCBI BLASTN (Altschul et al. 1990) and the EzBioCloud from the EzTaxon-e server (Kim et al. 2012) for bacterial identification. Comparative phylogenetic tree analysis was performed using the MEGA6 program with the neighbor-joining method (Tamura et al. 2013). All statistics were conducted using Microsoft Excel program (Microsoft, WA, USA), and *p* values <0.05 were considered significant.

Results

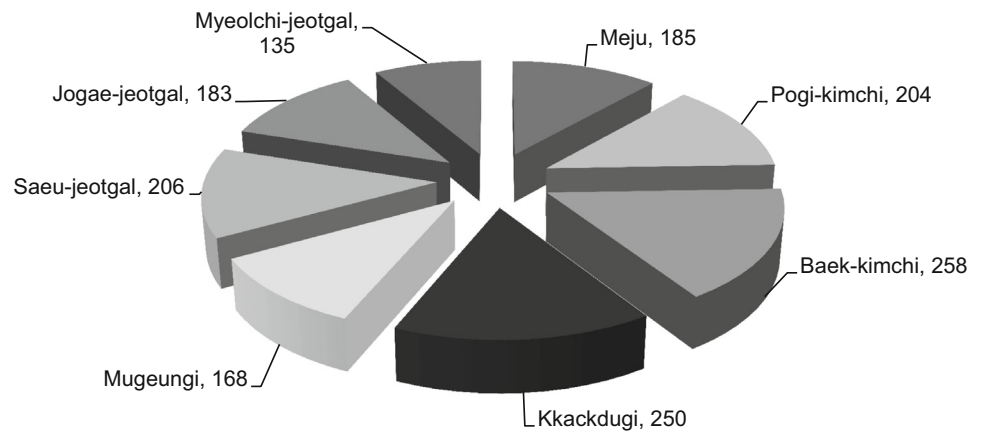
Isolation of fermenting bacteria

Using the selected four bacterial media under aerobic or anaerobic conditions, a total of 1589 fermenting bacteria were isolated from the collection of Korean fermented foods. The number of isolated fermenting bacteria was as follows: 258 strains from baek-kimchi, 204 from pogikimchi, 250 from kkakdugi, 168 from mugeungi, 206 from saeu-jeotgal, 183 from jogae-jeotgal, 135 from myeolchi-jeotgal, and 185 from meju (Fig. 1). To further elucidate the bacterial community from each fermented food, identification of the isolated bacteria was performed.

Grouping and identification of isolated fermenting bacteria using AP-PCR and 16S rRNA sequencing

The DNA fingerprinting method is generally useful for grouping the same or similar bacteria based on genomic DNA. For efficient identification of isolated fermenting bacteria, grouping was conducted using the AP-PCR method. After AP-PCR of all isolated fermenting bacteria, the 1238 bacterial strains were grouped into 219 groups based on identical AP-PCR band patterns. A few bacteria in each group were randomly picked, and their 16S rRNA genes were sequenced. Subsequent comparative analysis of 16S rRNA sequences with other bacterial 16S rRNA

Fig. 1 Bacterial isolates from eight Korean fermented foods



sequences in the GenBank database (Altschul et al. 1990) and EzTaxon server (Kim et al. 2012) revealed that all fermenting strains in the same group were identical and belonged to the same species. Identified bacterial AP-PCR band patterns in each fermented food sample are shown in Fig. S1. There were 351 strains that did not show any identical AP-PCR band patterns; as such, we were unable to group these strains. The 16S rRNA genes of each of the 351 strains were PCR-amplified and sequenced for bacterial identification. Results of the identification of the 16S rRNA sequences of all isolated fermenting bacteria and their source fermented foods are summarized in Table 1 (genus level) and Table S1 (species level).

Bacterial community analysis of Korean fermented foods

The most dominant genera in the eight Korean fermented foods were *Lactobacillus*, *Bacillus*, *Leuconostoc*, *Enterococcus*, *Weissella*, and *Staphylococcus* (Fig. 2 and Table 1). The major bacterial genera in the four kimchi samples (baek-kimchi, pogi-kimchi, kkakdugi, and mugeungi) were *Lactobacillus* (62.9, 35.0, 42.6 and 53.9 %, respectively), *Leuconostoc* (24.9, 23.5, 23.6 and 11.7 %, respectively), and *Bacillus* (9.0, 31.0, 15.7 and 24.0 %, respectively). *Weissella* was detected only in kkakdugi (14.5 %). The major bacterial genera shared by the three jeotgal samples (saeu-jeotgal, jogae-jeotgal, and myeolchi-jeotgal) were *Staphylococcus* (70.7, 9.8 and 43.5 %, respectively) and *Bacillus* (10.1, 4.4 and 6.9 %, respectively). However, each jeotgal sample had different dominant genera; *Lactococcus* (4.5 %) was dominant in saeu-jeotgal; *Weissella* (51.0 %), *Lactobacillus* (12.6 %), and *Leuconostoc* (12.0 %) in jogae-jeotgal; and *Pediococcus* (19.1 %) and *Micrococcus* (5.3 %) in myeolchi-jeotgal. Meju contained two major bacterial genera *Enterococcus* (46.7 %) and *Bacillus* (23.4 %) and two extra major genera, *Salmonella* (13.6 %) and *Staphylococcus* (9.8 %). Interestingly, while most major genera in Korean

fermented foods are lactic acid bacteria, *Staphylococcus* was also detected in all eight studied foods and was especially prominent in saeu-jeotgal and myeolchi-jeotgal. *Salmonella* was detected only in meju. The roles of these non-lactic acid bacteria in Korean fermented foods are not yet clearly understood.

Bacterial community analysis of ungrouped bacteria

Among the 1589 isolated fermenting bacteria, 351 strains did not show any identical AP-PCR band patterns and were not able to be grouped. Interestingly, subsequent 16S rRNA sequence analysis results showed that the bacterial community of the ungrouped bacteria was similar to that of the bacteria in 219 groups (Fig. 2). While their communities are similar even at the species level, why they have different AP-PCR band patterns even for the same species is still unknown. Nevertheless, among these ungrouped bacteria, unreported or even novel bacterial species may exist.

Prediction of novel species in ungrouped bacteria

16S rRNA sequences of all ungrouped bacteria were compared with those of the NCBI GenBank database and the EzTaxon server. The bacterial species with the lowest DNA sequence identity and coverage in each Korean fermented food are listed in Table 2. Any of these cultures could be a candidate for the discovery of a novel species. Among them, *B. cereus* from pogi-kimchi (99.72 % identity and 88.6 % coverage of 16S rRNA sequence), *L. curvatus* from baek-kimchi (99.52 % identity and 93.7 % coverage), and *W. thailandensis* from jogae-jeotgal (99.39 % identity and 94.6 % coverage) were selected for further characterization to confirm novel species. *W. thailandensis* FOL01 was analyzed using amplified random DNA restriction analysis (ARDRA), DNA–DNA hybridization for DNA–DNA relatedness (DDH), the API kit, VITEK analysis, GC content analysis, and cell-wall fatty acid analysis (S.-H. Lee, M.-J.

Table 1 The number of identified fermenting bacteria in eight Korean fermented foods

Bacterium	Baek-kimchi	Pogi-kimchi	Kkakdugi	Mugeungi	Saeu-jeotgal	Jogae-jeotgal	Myeolchi-jeotgal	Meju
<i>Bacillus</i>	20	62	38	37	20	8	9	43
<i>Bifidobacterium</i>	–	2	–	–	–	–	–	–
<i>Brevibacillus</i>	–	–	–	–	1	–	–	–
<i>Brevibacterium</i>	–	7	1	2	–	–	1	–
<i>Citrobacter</i>	–	–	–	–	–	1	–	–
<i>Corynebacterium</i>	–	–	–	–	–	–	4	–
<i>Enhydrobacter</i>	–	–	–	–	–	–	1	–
<i>Enterobacter</i>	–	–	–	3	–	6	–	–
<i>Enterococcus</i>	–	1	1	–	1	1	5	86
<i>Exiguobacterium</i>	–	–	–	–	1	–	–	–
<i>Kocuria</i>	–	–	–	–	2	–	–	–
<i>Lactobacillus</i>	139	70	103	83	5	23	7	–
<i>Lactococcus</i>	–	–	1	–	9	–	–	–
<i>Leclercia</i>	–	–	–	–	1	–	–	–
<i>Lelliottia</i>	–	–	–	–	–	1	–	–
<i>Leuconostoc</i>	55	47	57	18	2	22	–	7
<i>Lysinibacillus</i>	–	–	–	–	4	–	–	–
<i>Macrococcus</i>	–	–	–	–	3	–	2	–
<i>Micrococcus</i>	1	–	–	–	1	–	7	–
<i>Microbacterium</i>	–	–	–	–	1	–	–	–
<i>Paenibacillus</i>	–	–	–	–	–	–	2	–
<i>Pediococcus</i>	–	–	–	3	1	–	25	–
<i>Pluralibacter</i>	2	2	–	–	1	2	1	–
<i>Propionibacterium</i>	–	–	–	–	1	–	–	–
<i>Raoultella</i>	–	–	–	–	–	–	3	–
<i>Rummeliibacillus</i>	–	–	–	–	–	–	1	–
<i>Salmonella</i>	–	–	–	–	1	–	–	25
<i>Staphylococcus</i>	4	7	6	4	140	18	57	18
<i>Streptococcus</i>	–	2	–	–	–	–	–	–
<i>Vagococcus</i>	–	–	–	–	1	–	–	–
<i>Weissella</i>	–	–	35	4	2	101	6	5
Unidentified	37	4	8	14	8	–	4	1
Total	258	204	250	168	206	183	135	185

Ahn, J.-H Lee, unpublished data), substantiating that strain FOL01 does not belong to *W. thailandensis* but to a novel species of *Weissella*, designated *W. jogaejeotgali* (S.-H. Lee, M.-J. Ahn, J.-H Lee, unpublished data), supporting the prediction of novel species in ungrouped bacteria. Further identification and confirmation of novel species in ungrouped bacteria needs to be conducted for the detection of novel fermenting bacteria.

Discussion

Korean fermented foods, including kimchi, jeotgal, and meju, are known to be healthy and contain health-

promoting active compounds and lactic acid bacteria. While their bacterial communities have been studied using both culture-dependent and culture-independent methods, their roles in food fermentations are not yet clearly understood. The original objective of this study was the isolation of fermenting bacteria that produce health-beneficial compounds including bacteriocins and fibrinolytic enzymes. Eight Korean fermented foods were collected, and over 1500 fermenting bacteria were isolated from them. Subsequent DNA fingerprinting using AP-PCR revealed 219 bacterial groups. Identification of fermenting bacteria in each group using 16S rRNA sequencing revealed communities among the isolated fermenting bacteria from the eight Korean fermented foods. However, 351 strains were

Fig. 2 Bacterial communities of eight Korean fermented foods using a culture-dependent method. Bacterial composition of all isolated strains (a) and 351 ungrouped strains (b)

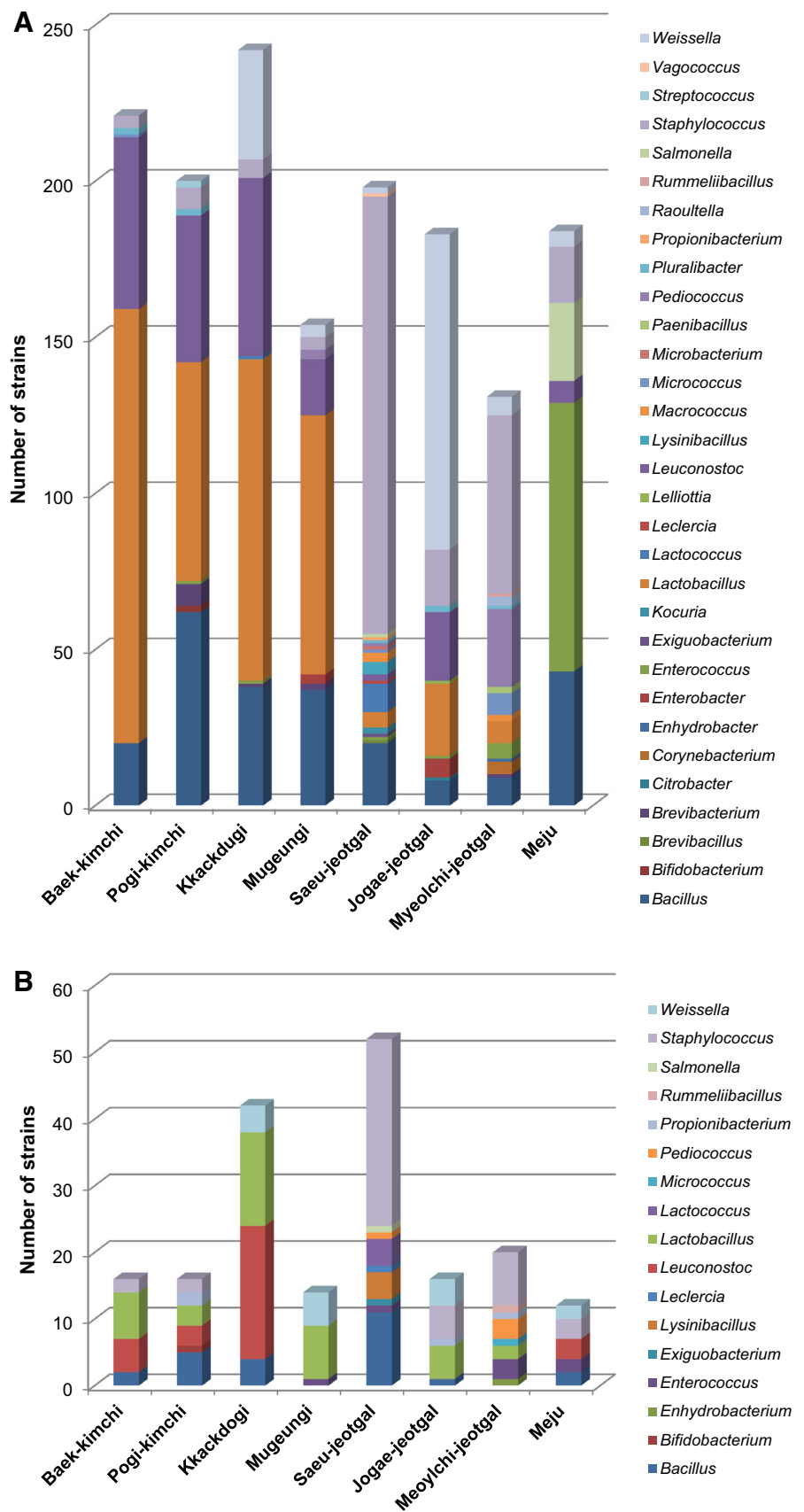


Table 2 Bacterial candidates considered as novel species

Food	Bacterial strain	Identification result	Identity (%)	Coverage (%)
Baek-kimchi		<i>Lactobacillus pentosus</i>	99.79	100
		<i>Lactobacillus curvatus</i>	99.52	93.7
Pogi-kimchi		<i>Bacillus cereus</i>	99.72	88.6
		<i>Streptococcus parasanguinis</i>	99.79	100
Kkakdugi		<i>Weissella confusa</i>	99.77	100
		<i>Bacillus halosaccharavorans</i>	99.77	97.6
Mugeungi		<i>Lactobacillus gaminis</i>	99.87	100
		<i>Bacillus tequilensis</i>	99.87	100
Saeu-jeotgal		<i>Enterococcus faecium</i>	99.5	100
		<i>Weissella thailandensis</i>	99.4	94.6
		<i>Brevibacterium permense</i>	99.78	99.5
Jogae-jeotgal	FOL01 ^a	<i>Weissella thailandensis</i>	99.39	94.6
		<i>Lactobacillus curvatus</i>	99.73	93.7
		<i>Staphylococcus warneri</i>	99.77	99.7
		<i>Lactobacillus rhamnosus</i>	99.75	99.8
Meju		<i>Weissella cibaria</i>	99.54	100

^a This strain was identified to belong to a novel species, *W. Jogaejeotgali* (S.-H. Lee, M.-J. Ahn, J.-H. Lee, unpublished data)

not able to be grouped because they did not show identical AP-PCR banding patterns with grouped fermenting bacteria. Identification of each type of fermenting bacteria and its bacterial community analysis showed that the bacterial composition of each Korean fermented food among the ungrouped 351 strains was not that different from the bacterial community of grouped fermenting bacteria for each fermented food, suggesting that the bacteria belonging to the same species could have different DNA fingerprinting results.

Bacterial community analysis showed that the major bacteria in fermented foods are very similar at the genus level but very different at the species level. For example, the most dominant fermenting bacteria in kimchi foods (baek-kimchi, pogi-kimchi, kkakdugi, and mugeungi) are *L. curvatus*, *L. sakei*, and *L. mesenteroides* (Table S1), which is different from previous 16S rRNA profile reports showing *L. plantarum* and *W. koreensis* as the most dominant fermenting bacteria in kimchi (Kim and Chun 2005; Jung et al. 2011). In meju, we detected *E. faecium* and *B. pumilus* as the major meju-fermenting bacteria (Table S1), which is quite different from previous 16S rRNA profile results showing *E. durans*, *B. siamensis*, and *B. sonorensis* (Kim et al. 2011b, 2011c). The different identification may be due to sequencing of short 16S rRNA gene PCR products using 340F/758R (~400-bp) (Juck et al. 2000), which are different from our 16S rRNA gene PCR products (580-, 890-, or even 1,450-bp) (Ku and Lee 2014; Lane et al. 1985; Choi et al. 2013) with higher resolution and identification accuracy, suggesting that bacterial identification using sequencing of short 16S rRNA gene PCR products may not be correct at the species level. To overcome this problem, both the culture-

dependent method with a high number of sample bacteria and 16S rRNA profiling analysis with a long-range 16S rRNA universal primer set may be used to enhance identification resolution and accuracy. This 16S rRNA profiling analysis can provide bacterial community information of culturable as well as unculturable bacteria. Previous culture-dependent studies were conducted with a low number of bacterial isolates per sample (generally <100 strains), which is not enough to represent the whole bacterial community in a food sample (Kim et al. 2011b).

The most dominant species in saeu-jeotgal in this study were *S. equorum*, *S. saprophyticus*, and *S. warneri* (Table S1). Previous bacterial community studies of saeu-jeotgal also showed *S. equorum* as the most dominant strain in this food (An and Lee 2011). This strain is highly halophilic and is believed to contribute to enhancing the fermentation flavor, suggesting that it may be an appropriate candidate as a saeu-jeotgal starter culture (An and Lee 2011). However, our bacterial community analysis of jogae-jeotgal revealed that 51.0 % of the total isolated strains were *Weissella* and that myeolchi-jeotgal had *Staphylococcus* (43.5 %) and *Pediococcus* (19.1 %) as its major genera. These species are probably involved in fermentation flavor (Table S1).

In general, when DNA–DNA relatedness is less than 70 % or 16S rRNA sequence identity is less than 97 %, the strain is accepted as a novel species (Wayne et al. 1987; Stackebrandt and Goebel 1994). In this study, we could not find any isolated fermenting bacterium with <97 % 16S rRNA sequence identity. However, further bacterial identification and characterization of the isolated strain FOL01 (99.39 % 16S rRNA sequence identity and 63.9 % DNA–DNA relatedness) revealed that it belongs to a novel

species, *W. jogaеjeotgali* (S.-H. Lee, M.-J. Ahn, J.-H. Lee, unpublished data), suggesting that the general parameters for determination of a novel species may be not always correct (Table 2). Therefore, although 16S rRNA sequence identity is more than 97 %, further identification and characterization testing including DNA–DNA relatedness must be conducted to confirm whether or not the isolated strain belongs to a novel species.

While most studies for bacterial communities in fermented foods have been conducted using a metagenomic approach, the culture-dependent method with a number of isolated fermenting bacteria may be still valuable for enhancement of bacterial identification accuracy. This study extends our understanding of Korean fermented foods and their bacterial communities. Our results provide important information about the roles and functional properties of fermenting bacteria in the production of Korean fermented foods.

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