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Review

Starter Cultures for Kimchi Fermentation

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Kimchi is a traditional Korean vegetable product that is naturally fermented by various microorganisms present in the raw materials. Among these microorganisms, lactic acid bacteria dominate the fermentation process. Natural fermentation with unsterilized raw materials leads to the growth of various lactic acid bacteria, resulting in variations in the taste and quality of kimchi, which may make it difficult to produce industrial-scale kimchi with consistent quality. The use of starter cultures has been considered as an alternative for the industrial production of standardized kimchi, and recent trends suggest that the demand for starter cultures is on the rise. However, several factors should be carefully considered for the successful application of starter cultures for kimchi fermentation. In this review, we summarize recent studies on kimchi starter cultures, describe practical problems in the application of industrial-scale kimchi production, and discuss the directions for further studies.

Keywords: Kimchi, starter culture, lactic acid bacteria, fermentation control

Introduction

Kimchi is a traditional Korean food product made by fermenting vegetables, such as salted cabbage, radish, and cucumber, with various spices, including red pepper powder, garlic, ginger, and other ingredients. The addition of salted and fermented seafood products and other seasonings is optional [10]. Kimchi is fermented by lactic acid bacteria (LABs) at low temperatures, ensuring proper ripening and preservation [3]. The production of organic acids from carbohydrates and the resulting reduction in pH maintain the freshness of the vegetables during storage. LABs produce various compounds in addition to organic acids, including CO_2, ethanol, mannitol, bacteriocins, γ-aminobutyric acid (GABA), ornithine, conjugated linoleic acids, and oligosaccharides; these components contribute to the fermentation characteristics and health functionality of kimchi [8, 12, 26, 27, 45, 54, 60]. Properly fermented kimchi is flavorful, possessing a distinct, savory combination of sour, spicy, hot, sweet, and fresh tastes. The fermentation is markedly affected by environmental factors, such as temperature, salt concentration, and the presence of certain ingredients [34, 50, 61, 62].

Aerobes initially increase during the early stages of kimchi fermentation, while anaerobes (mostly LABs) increase steadily throughout the middle stages of fermentation. LABs such as *Leuconostoc* spp., *Lactobacillus* spp., and *Weissella* spp. are the main microbial flora involved in this process [26, 44, 55]. Previous studies have shown that *Leuconostoc mesenteroides* is the major microorganism present during the optimal ripening period of kimchi at low temperatures (usually <10°C); *Lactobacillus plantarum* becomes the predominant species during the later stages and is regarded as an undesirable organism responsible for acidic deterioration at temperatures greater than 10°C and the subsequent reduction in kimchi quality [40, 41, 50, 63]. Additionally, *Lb. sakei* is the most abundant LAB in over-ripened kimchi and, along with *Lb. plantarum*, is considered responsible for the over-ripening and reduced quality of kimchi [48]. The genes related to lactic acid production in *Leu. mesenteroides* are primarily expressed during the early fermentation stage, whereas the corresponding genes in *Lb. sakei* are actively expressed during the middle and late fermentation stages [25].

The bacterial communities present during the fermentation of kimchi were previously analyzed by culture-dependent identification methods based on the morphologic and phenotypic characteristics of cells grown on agar culture

media [13, 29, 35]. More recently, culture-independent methods, such as polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis (DGGE) and PCR-pyrosequencing based on the direct amplification of the 16S rRNA gene, have provided a more accurate means of species identification [26, 44]. To date, *Leu. citreum*, *Leu. gasicomitatum*, *Leu. carnosum*, *Leu. gelidum*, *Leu. mesenteroides*, *Lb. sakei*, *Weissella koreensis*, and *W. cibaria* have been found to be the predominant microbes in several kimchi samples, whereas members of the genera *Lactococcus* and *Pediococcus* have been detected as minor populations [13, 25, 26, 29, 39, 44]. Moreover, a large number of phage DNA sequences were detected during kimchi fermentation in a recent study, suggesting that a high proportion of LABs are infected by bacteriophages, which may be an important determinant of kimchi fermentation [24, 26].

For the manufacturing of commercial kimchi products, natural fermentation with unsterilized raw materials leads to the growth of various LABs, which makes it difficult to control the fermentation process. This natural fermentation often results in end products of inconsistent quality. The use of starter cultures has been considered as an alternative to address these outstanding problems. The purpose of applying starter cultures to kimchi includes improving the sensory characteristics, extending shelf-life, and achieving functional properties and uniform quality [8, 23, 27, 48]. To date, *Leu. mesenteroides* DRC0211 has been used as a starter culture for commercial kimchi production, and recent trends suggest that the demand for starter cultures is on the rise [1, 55]. However, there are many limitations to the use of kimchi starters. For example, commercialized starter cultures are difficult to find, and the unit price of the kimchi product is increased when such starter cultures are used.

This review aims to summarize the characteristics of kimchi starter cultures that have been studied to date, discuss the drawbacks of industrial-scale kimchi applications, and describe future research directions to resolve these drawbacks.

Selection Criteria and Application of Starter Cultures for the Production of Kimchi

Starter cultures often include a large number of various microorganisms that accelerate and improve the fermentation process [14, 19]. Microorganisms selected for this purpose are expected to possess specific characteristics, including the ability to adapt to the fermentation environment. Starter cultures used in kimchi need to adapt well to the unique environment of kimchi fermentation, which includes low temperature, low pH, and the presence of NaCl [33, 48, 64]. Homemade and commercially prepared kimchi products are typically stored under refrigerated conditions, and the organoleptic quality of kimchi fermented at low temperatures (usually <10°C) is superior to that of kimchi fermented at room temperature [64]. Therefore, the LABs used in kimchi constantly face a low-temperature challenge, and as such, an important characteristic of starter cultures is the ability to thrive at low temperatures [11]. As kimchi fermentation progresses, the pH decreases, while the titratable acidity increases as a result of acids produced by LABs. The pH decreases to below 4.0 when kimchi is overfermented. The presence of NaCl (1%−4%) in kimchi represses the growth of some undesirable spoilage microorganisms and provides favorable conditions for the growth of LABs [3, 24].

Studies on kimchi starter cultures have mainly focused on the production of kimchi with extension of the optimal ripening period, improved sensory properties and functionality, and enhanced safety by using predominant LABs in kimchi fermentation, acid-resistant strains inhibiting overacidifying microorganisms, and bacteriocin-producing strains (Table 1, Fig. 1).

Improvement of Sensory Characteristics

Leuconostoc spp. are widely used as kimchi starters because these strains are expected to have favorable effects on kimchi fermentation, including are improvement of sensory characteristics [13, 22, 27]. Previous studies have reported that *Leuconostoc* spp. are dominant from the initial to middle stages of kimchi fermentation. When *Leu. mesenteroides* B1 is used as a starter, the consumption of free sugar begins earlier and results in slightly increased production of lactic acid, acetic acid, and mannitol, demonstrating that kimchi fermentation using a starter is completed earlier, with more production of kimchi
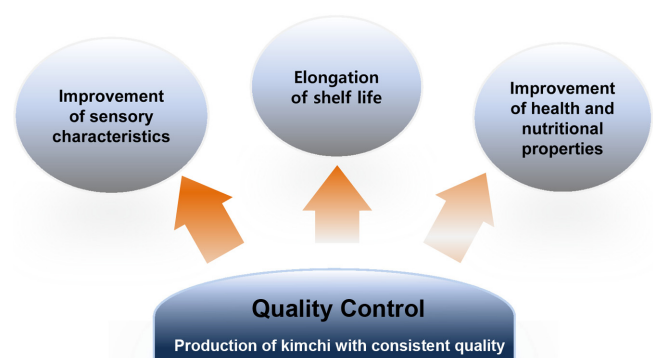
Fig 1. Use of starter cultures for kimchi fermentation.

Table 1. Characteristics of starter cultures and effects on kimchi fermentation.

Starter cultures	Characteristics	Effects on kimchi	References
Lb. plantarum HSU015, *P. cerevisiae* HSU02, *Lb. brevis* HSU01, *Leu. mesenteroides* HSU05	Rapid fermentation	Acceleration of the fermentation process	[49]
Leu. mesenteroides subsp. *mesenteroides* A02, *Leu. mesenteorides* subsp. *dextranicum* A18, *Leu. paramesenteroides* B30, *Lb. bavaricus* B01, *Lb. homohiochii* B21	Psychrotrophic lactic acid bacteria	Acceleration of the fermentation process	[64]
Leu. paramesenteroides as an acid-resistant mutant	Acid resistance	Inhibition of *Lb. plantarum*	[37]
Mutant strain of *Leu. mesenteroides* and *Leu. paramasenteroides*	Adipic acid resistance	Delayed acidification and higher in total acceptability	[43]
Leu. citreum IH22	Predominant lactic acid bacteria involved in kimchi fermentation	Maintained kimchi quality for prolonged periods	[13]
Bifidobacterium spp.	Production of conjugated linoleic acid (CLA)	Increased CLA contents	[51]
B. longum BO-11	Tolerance to acid and bile	Improvement of functionality	[5]
Leu. mesenteroides K2M5, *Lb. sakei* K5M3	Predominant lactic acid bacteria involved in kimchi fermentation	Maintained kimchi quality for prolonged periods	[22]
Leu. citreum GJ7	Production of bacteriocin	Prevented over-ripening and extended shelf-life	[8]
Lb. plantarum PL62	Production of conjugated linoleic acids with anticancer and anti-obesity activities	Improvement of functionality	[45]
Leu. citreum, Lb. plantarum	Predominant lactic acid bacteria involved in kimchi fermentation	Improvement of functionality	[17]
Leu. mesenteroides strain B1	Production of mannitol	Shortened the time to reach optimal ripened state	[27]
Leu. mesenteroides LK93	Resistance to acid and bile salts; antimicrobial and antifungal activities	Inhibition of the growth of film-forming yeast	[57]
Lb. plantarum 3099K, *Lb. plantarum* pnuK, *Leu. mesenteroides* pnuK	Antioxidant and anticancer activities	Improvement of functionality	[2]
Leu. citreum KACC91035	High dextransucrase activity	Improvement of isomaltooligosaccharide production	[12]

metabolites than in fermentation without a starter culture [27]. Based on studies examining the major microbial composition of kimchi and its association with taste, *Leu. mesenteroides* K2M5 has been selected as a starter to improve the sensory properties and control the microbial composition [22]. Since 2004, *Leu. mesenteroides* DRC has been used as a starter culture in the Korean kimchi industry for achieving greater organoleptic effects [1].

In addition to *Leu. mesenteroides*, other *Leuconostoc* spp. have also been reported as dominant species according to fermentation temperature. *Leu. gelidum* and *Leu. citreum* have been shown to be dominant species during the early fermentation stage of kimchi fermented at 8°C and 15°C, respectively, while *Leu. mesenteroides* was found to be a minor species [29]. Moreover, after inoculation in kimchi, *Leu. citreum* strain IH22 has been shown to dominate the culture and retard the growth of other LABs in kimchi fermented at 15°C, suggesting that this microbe could be used as a starter culture to maintain the kimchi quality for prolonged periods [13].

Control of Fermentation Rate

To hasten the fermentation process, *Leu. mesenteroides* HSU05, *Lb. plantarum* HSU015, *Lb. brevis* HSU01, and *Pediococcus cerevisiae* HSU02 have been used in starter cultures at a ratio of 1:1:1:1; this shortens the time required to reach the optimal fermentation stage by about 24 h compared with that of control kimchi at 25°C; moreover, the use of mixed strains is more effective than the use of a single strain [49]. Five strains of psychrotrophic LABs (*Leu. mesenteroides* subsp. *mesenteroides* A02, *Leu. mesenteroides* subsp. *dextranicum* A18, *Leu. paramesenteroides* B30, *Lactococcus bavaricus* B01, and *Lc. homohiochii* B21) inoculated as kimchi starters shorten the time required to reach an optimally ripened state at 8°C. In kimchi not inoculated with a starter, 10 days are required to reach the optimal ripened state; in contrast, all starter-inoculated kimchi samples require only 4 days to reach the optimal ripened state [64]. However, although it is clear that the time to reach the optimal ripened state can be shortened, the development of technologies for the long-term maintenance of quality after reaching the optimal ripening period through rapid fermentation has not been considered.

Extension of Shelf-Life

Although *Leuconostoc* is an early predominant species, it has the disadvantage of low applicability as a starter owing to weak acid resistance [28, 39]. Therefore, to compensate for this shortcoming, strains with acid resistance have been selected or acid resistance has been enhanced through mutation, thereby improving the applicability of the strain as a starter. Resistance to acid can help to maintain the role of starters during kimchi fermentation, because kimchi finally reaches the acidified state (around pH 4.0). Some studies have shown that kimchi fermentation is completed with higher scores in sensory evaluation, and the growth of *Lactobacillus* spp. is inhibited when acid-resistant mutants are used as a starter, contributing to the extension of shelf-life and improved sensory characteristics [28, 36–39, 43]. Low pH is a growth-limiting condition for *Leu. mesenteroides* during kimchi fermentation; this limitation can be overcome by using acid-resistant mutant strains obtained after nitrosoguanidine treatment, which could grow at low pH (around pH 4.0). Kimchi inoculated with the mutant strain M-10 is superior in sensory evaluation and has a prolonged optimal ripening period during fermentation at 10°C [28]. The adipic acid-resistant mutant strains *Leu. mesenteroides* and *Leu. paramesenteroides*, with adipic acid, have been used to suppress the growth of acidifying microorganisms during kimchi fermentation, effectively enhancing the organoleptic characteristics and extending the shelf-life of the kimchi product [43].

Yeasts present at later stages of fermentation can produce various tissue-softening enzymes, including polygalacturonase, which destroys pectic substances and other structures in cabbage and radish tissues that undermine kimchi quality [6]. *Leu. mesenteroides* LK93 having antimicrobial and antifungal activities showed the ability to inhibit the growth of film-forming yeast [57]. However, yeasts, which use lactic and acetic acids as carbon sources, have also been used as starter cultures to reduce the abundance of overproduced organic acids during the later stages of kimchi fermentation [31, 32]. The results showed that *Saccharomyces* spp. prolong the shelf-life by reducing the production of organic acids and improving the fresh flavor of kimchi. Furthermore, the co-inoculation of yeast with the acid-resistant mutant *Leu. mesenteroides* M-100 could have a synergistic effect in terms of the retardation of acidification of kimchi owing to low production of lactic acid, lengthen the edible period by reducing the levels of lactic acid and acetic acid by *Saccharomyces fermentati*, and improve the overall acceptability of the sensory properties of kimchi [38].

Many studies have examined the applicability of bacteriocin-producing strains as kimchi starters [8, 9, 52, 57]. These studies have mainly focused on the extension of the optimal ripening period by inhibiting kimchi acidifying microorganisms or suppressing harmful microorganisms that can contaminate the early kimchi fermentation process. Efforts to extend the shelf-life of kimchi have been attempted by the inhibition of *Lb. plantarum* using bacteriocin-producing LABs. Moreover, successful extension of the shelf-life of kimchi was reported using *Enterococcus* sp. bacteriocin-producing strains isolated from kimchi as a starter [52]. However, the use of *Enterococcus* as a starter is considered inappropriate owing to safety issues, as these bacteria contribute to the emergence of antimicrobial resistance [18]. In addition, the bacteriocin-producing *Leu. mesenteroides* and *Leu. citreum*, which inhibit the growth of *Lb. plantarum*, have been identified, and their bacteriocin-producing characteristics have been elucidated [7, 65]. Among these bacteria, the application of *Leu. citreum* GJ7 to kimchi as a starter culture has resulted in improved sensory characteristics and extended shelf-life [8]. *Leu. citreum* GJ7 has also been shown to inhibit the growth of *Escherichia coli* and gram-negative bacteria during kimchi fermentation [9]. Besides bacteriocin-producing LABs, *Leuconostoc* and *Weissella* species, which produce nonproteinaceous antibacterial substances that inhibit *Lb. sakei*, one of the most abundant LABs in over-ripened kimchi, have been isolated for future application as starter

cultures in kimchi fermentation [33, 48]. In a recent study, *Lc. lactis*, which has antibacterial activity against bacteria associated with overacidification of kimchi, including *Lb. plantarum*, *P. pentosaceus*, and *Lb. sakei*, was shown to have potential as a starter culture to extend the shelf-life of kimchi [11].

Improvement of Functionality

To promote the functional properties of kimchi, many researchers have examined the use of kimchi starter cultures. Some studies have shown that inoculating kimchi with starter cultures could provide health benefits *via* increased antioxidant and anticancer activities [2]. Starter cultures producing GABA and anti-obesity materials have also been used. Some LABs isolated from kimchi have been shown to catalyze the decarboxylation of glutamate, releasing GABA and CO_2 as end-products [60]. *W. koreensis* OK1-6 isolated from kimchi exhibits excellent ornithine-producing capacity and is highly effective at inhibiting intracellular lipid accumulation in differentiating adipocytes [54, 56]. Moreover, kimchi fermented with *Leu.* kimchi GJ2 shows cholesterol-lowering effects in rats consuming a high-fat and high-cholesterol diet [23].

Bifidobacterium spp. have been used to improve the functionality of kimchi because of their ability to survive during fermentation, although there are factors that prevent the predominance of these bacteria in the kimchi fermentation [4, 5, 51].

A new strategy that exploits an enzymatic reaction of starter cultures has been proposed for increasing oligosaccharide production and maintaining an appropriate level of sweetness in kimchi [12, 66]. The LAB *Leu. citreum* KACC 91035, which expresses highly active glycosyltransferases, has been used for the synthesis of beneficial oligosaccharides in kimchi. In addition, the addition of a sucrose-maltose combination, added as donor and acceptor, with the starter culture has been shown to confer an appropriate level of sweetness to the product by releasing fructose and preventing unfavorable polymer synthesis *via* isomaltooligosaccharide production.

There is ample evidence supporting the potential health benefits of using starter cultures; however, these studies have primarily been performed using *in vitro* or mouse models. Therefore, clinical tests are needed to determine whether these results are applicable to humans [24].

Initial Inoculum Levels and Monitoring of Starter Cultures

Different varieties of microorganisms are present in raw materials and initiate kimchi fermentation. One important characteristic of a starter culture is its ability to predominate over native microorganisms present in raw materials. This depends on rapid growth under fermentation conditions and/or the ability to produce antagonistic substances, such as bacteriocin. The initial number of inoculated bacteria is a critical factor in determining whether the starters can predominate in the fermentation environment. Indeed, the majority of studies have examined starter cultures with more than 100-times as much starter (7−8 log CFU/g) as the initial LAB counts (4−5 log CFU/g) typically present in the early stages of kimchi production. Additionally, the initial inoculum level of kimchi starters is thought to be a crucial factor influencing the manufacturing unit price and the effects on kimchi fermentation. However, these factors have not been described in depth. Therefore, further studies are required to determine the optimal inoculum level of kimchi starters and to reduce the initial microbial counts in kimchi raw materials.

When starter cultures are inoculated, they are typically mixed with the microflora present in the raw materials. For clarifying the starter growth in kimchi and for predicting starter predominance during fermentation, it is necessary to monitor the starter in natural or inoculated flora [15]. The growth of starter cultures has been monitored by cell counts, microscopic observation, and molecular techniques such as PCR-DGGE, random gene integration using transposons, barcoded pyrosequencing, and PCR with specific primer sets (Table 2). However, it is not easy to discriminate starter cultures from native flora in kimchi because strains isolated from kimchi are typically used as starter cultures. A *Leuconostoc* mutant generated by transposon-mediated genomic integration of the chloramphenicol resistance gene (*cat*) has been shown to be useful for monitoring the growth of the starter strain [15]. Based on these previous works, a standardized method for determining starter culture counts may need to be established in order to effectively monitor their growth and survival.

Pretreatment of the Kimchi Ingredients

The population of microorganisms present in raw materials is important for determining the kimchi microflora succession, because kimchi is generally processed by natural fermentation without any sterilization processes [20]. Therefore, it is important to reduce the initial microbial counts in the raw materials in order to maximize the efficiency and maintain the activity of starter cultures added during kimchi fermentation. Thus, the pretreatment of raw ingredients to

Table 2. Initial inoculum levels and monitoring of starter cultures used for kimchi fermentation.

Kimchi varieties	Starter cultures	Initial no. of LAB Control kimchi /Starter kimchi	Monitoring methods	References
Cabbage kimchi	*Leu. mesenteroides* DRC	-	Random gene integration method	[15]
Cabbage kimchi	*Leu. citreum* GJ7	4.2 log CFU/ml 7.6 log CFU/ml	Microscopic observation & API 50CHL kit PCR	[8]
Cabbage kimchi	*Lb. plantarum* PL62	4.0×10^6 CFU/ml 1.8×10^8 CFU/ml	PCR with group-specific primers/DGGE	[45]
Cabbage kimchi	*Leu. citreum* GJ7	3.43 log CFU/ml 7.50 log CFU/ml	Microscopic observation API 50CHL kit PCR	[9]
Cabbage kimchi	*Leu. citreum* KM20/ *Lb. plantarum* KCTC 3099	5 log CFU/ml 4~4.5 log CFU/ml	Microbial count using medium	[17]
Cabbage & radish kimchi	*Leu. mesenteroides* B1	- 7 log CFU/g	Barcoded pyrosequencing	[27]
Cabbage kimchi	*Leu. mesenteroides* LK93	3.63 log CFU/ml 7.85 log CFU/ml	16S rDNA sequencing	[57]
Dongchimi	*Leu. citreum* KACC 91035	5 log CFU/ml 7 log CFU/ml	PCR-DGGE analysis	[12]

reduce the initial number of microorganisms can significantly affect the product characteristics, especially when starter cultures are used. However, most studies investigating the reduction of initial microbial counts in raw materials have been conducted to improve the safety and shelf-life of kimchi rather than for the starter culture application [17, 46, 47]. For example, contaminating microbes on the raw materials can be substantially reduced by ozone (up to 6 ppm) or gamma irradiation (up to 5 kGy) without affecting the nutritional content, and kimchi produced from sanitized materials shows little change in terms of microbial population but exhibits a longer storage time [46, 47]. Unfortunately, these methods do not meet consumer standards for food safety and have not been widely used in the food industry.

The addition of mixed starter cultures containing *Leu. citreum* and *Lb. plantarum* to pasteurized brined cabbage was shown to significantly increase LAB counts, and the fermentation patterns were shown to be similar to those of naturally fermented kimchi. However, since most of the raw materials are heat-sensitive, more studies on new technologies to reduce initial microbial loads in raw materials are necessary for the starter cultures to be used effectively [17, 58].

Safety Assessment of Starter Cultures

Despite the importance of carrying out safety assessments prior to the application of starters to commercial kimchi, the safety assessments have rarely been performed in studies of kimchi starter development [22, 42]. In one of the two studies on this topic reported to date, *Leu. mesenteroides* K2M5 and *Lb. sakei* K5M3, both of which are used as kimchi starters, did not show harmful characteristics, such as β-hemolysis, ammonia and indole formation, and gelatin liquefaction, and did not exhibit activities of several harmful enzymes, including phenylalanine deaminase, β-glucuronidase, β-glucosidase, 7α-dehydroxylase, and nitroreductase [22]. Additionally, a recent study assessed the antibiotic resistance and biogenic amine production of *Lactobacillus* spp. strains isolated from kimchi [42].

Recently, many studies have reported whole-genome sequencing results of a variety of LABs isolated from kimchi (Table 3) [21]. These results will provide insight into genotype safety properties involving retention of antibiotic resistance genes and toxin-producing genes.

Mass Production of Kimchi Starter Cultures

Mass production is required for the industrial utilization of kimchi starter cultures, and a variety of formulation techniques are needed for its stable distribution [16, 53]. The cultivation process, choice of protective agents, and stabilization technique are important variables. Biomass production is normally followed by the stabilization of cells by cryoconcentration and freeze-drying. To improve the viability of freeze-dried LABs, various protective agents have been used. Compared with skim milk, 10% garlic

Table 3. Genome sequence features of lactic acid bacteria isolated from kimchi (modified from Jang and Kim [21]).

Strains	GenBank Accession No.	Chromosome size (Mb)	GC content (%)	Year published
Leu. citreum KM20	DQ489736	1.80	39.0	2008
Leu. kimchi*i* IMSNU11154	CP001758	2.01	37.9	2010
Leu. argentinum KCTC 3773	AEGQ00000000	1.72	42.9	2010
Lb. plantarum ST-III	CP002222	3.25	44.5	2011
Leu. fallax KCTC 3537	AEIZ00000000	1.64	37.5	2011
W. cibaria KACC 11862	AEKT01000000			2011
Leu. gelidum KCTC 3527	AEMI00000000			2011
Lb. coryniformis subsp. *coryniformis* KCTC 3167	AELK00000000	2.96	42.8	2011
Lb. animalis KCTC 3501	AEOF00000000	1.88	41.1	2011
Leu. inhae KCTC 3774	AEMJ00000000			2011
Lb. farciminis KCTC 3681	AEOT00000000	2.50	36.4	2011
W. koreensis KACC 15510	CP002899	1.42	35.5	2011
Leu. kimchi*i* strain C2	CP002898	1.88	37.9	2011
Leu. mesenteroides subsp. *mesenteroides* strain J18	CP003101	1.90	37.8	2012
W. koreensis KCTC 3621T	AKGG00000000	1.73	35.5	2012
Leu. gelidum JB7	CP003839	1.89	36.68	2012
Leu. carnosum JB16	CP003851	1.64	37.24	2012
P. pentosaceus SL4	CP006854	1.79	37.3	2013
Lb. plantarum Wikim18	JMEL00000000	3.35	44.3	2014

paste shows suitable cryoprotective effects [59]. Recently, the viability of freeze-dried LABs, including *W. cibaria*, *Lb. plantarum*, *Lb. sakei*, and *Leu. citreum*, was evaluated with food-grade protective agents (*e.g.*, skim milk, yeast extract, soy powder, and trehalose). Soy powder was shown to be the best among the protective agents examined, maintaining approximately 90% viability of LABs during the freeze-drying process [16].

In order to supply starter cultures to kimchi factories at a low cost, LAB cultivation processes using MFL medium containing cabbage extract, maltose, yeast extract, and inorganic salts have been studied. The number of *Leu. citreum* GR1 cultivated in MFL medium was 2.2 times higher than that obtained in standard MRS medium [53]. Additionally, the acid stress resistance of *Leu. mesenteroides* could be improved by cultivation in MRS medium supplemented with glutathione, which is involved in resistance to acid, oxidative, and osmotic stress [30].

Concluding Remarks and Future Perspectives

In order to meet the increasing global demand for kimchi, it is necessary to develop reliable methods that yield a stable and high-quality product. The best approach involves inoculating optimized starter cultures and allowing fermentation

to proceed in a controlled way. Most commercially produced kimchi still rely on natural fermentation; however, the prospective use of starter cultures has become more attractive to the industry owing to improvements in the organoleptic characteristics, safety, health benefits, and shelf-life of kimchi. Several starter cultures for kimchi have been shown to inhibit microorganisms related to over-ripening of kimchi and food-related pathogens. Moreover, starter cultures have been shown to produce much more lactic acid, acetic acid, and mannitol from free sugars during the initial stage, ultimately accelerating the fermentation process. In addition, many studies have shown that kimchi inoculated with starter cultures has better health-promoting properties, such as antioxidant, anticancer, and anti-obesity effects, compared with conventional kimchi. More oligosaccharides and GABA are also produced in starter kimchi. However, some studies have indicated that the use of starter cultures is associated with incomplete control of the entire process of kimchi fermentation; these studies have generally been performed over a short duration; that is, not sufficiently long enough to evaluate the extension of kimchi shelf-life.

At present, the availability of genomic sequences for many organisms has also allowed researchers to rapidly discern the metabolic potential of specific strains. Indeed, the sequenced genomes of many LABs isolated from

kimchi are now available and are likely to provide useful information for optimizing starter cultures.

Although many studies have examined the use of starter cultures for kimchi fermentation, a clear picture of the applicability of this method has not fully emerged. Indeed, few starter cultures are actually being used in the industrial production of kimchi, and some practical problems still remain to be solved to improve the quality of kimchi using starter cultures. The problems in commercial kimchi production using starters include (i) lack of various types of commercialized starter cultures, (ii) increases in the unit price of kimchi production due to starter addition, (iii) the absence of guidelines for the safety assessment of kimchi starter cultures, and (iv) lack of developed methods to reduce initial microbial counts in raw materials. In addition, further research on phage-host interactions is needed to assess the contribution of LAB succession to kimchi fermentation, which could help to optimize the preservation period and prevent the over-ripening of kimchi, facilitating the development of phage-resistant kimchi starter cultures.

Currently, the industrial-scale production of manufactured kimchi has increased compared with the production of homemade kimchi. Therefore, more research efforts will be required to accelerate improvements in the kimchi quality, particularly in the context of industrial-scale production. Therefore, studies on kimchi starter cultures should aim to resolve these issues.

Acknowledgments

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