Detection and rRNA Gene-Based Identification of the Major Lactic Acid Bacteria Isolated from the Fermenting Kimchi

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Introduction

Fermented vegetables are consumed worldwide as sauerkraut, pickled cucumber and olive, zukemono(Japan), and kimchi(Korea) and so forth, where lactic acid bacteria(LAB) play a crucial role in their fermentation processes(Chiegh and Park, 1994). For kimchi preparation, oriental cabbage or radish is mostly used as raw materials, but many other vegetables are also brined for the preparation of a variety of kimchi. Reportedly more than 200 kinds of kimchi have been produced in Korea depending on the raw materials used, processing methods, seasons, and geographical locations (Ku and Choi, 1991). With the socioeconomic change along with urbanization in Korea, the trend consuming mass-produced kimchi rather than the household preparation is ever increasing so that the food scientists have lots of interests on microorganisms affected to the taste and the quality of final product. According to a national nutrition survey, an adult consumes 50-100 g of kimchi daily in summer and 150-200 g in winter. The consumption of commercial products increases yearly by 15-20%. Since the flavor of fermented food is attributed largely to the characteristic fermentation and ripening processes, and the role of the microorganisms involved. Of the many factors that can affect kimchi fermentation, the type of microorganisms that dominate the process often dictates the final quality. It was previously reported that the best taste was obtained at pH 4.2 and 0.6% lactic acid in the acidity three days after fermentation under 3% NaCl brine at 20℃ (Mheen and Kwon, 1984). Generally natural populations of LAB on plant material are often low in number and heterofermentative in their sugar utilization pattern. As it has been long believed, heterofermenters such as *Leu. mesenteroides* is predominant in the initial stage and homofermentative strains representing *Lb. plantarum* is main flora in the final fermenting stage of kimchi (Mheen and Kwon,1984; Lee et al., 1992). Despite of extensive researches on this issue past 50 years, limited information is available on the microbial ecology of fermenting kimchi, especially with regard to the ecological studies on LAB using modern molecular typing during the fermentation process.

PCR assays based on genes or intergenic regions of the rRNA locus have been very useful for the environmental detection of both prokaryotic and eukaryotic microorganisms (Roggrigues et al., 1991). Because the rRNA genes are tandemly repeated in high copy numbers (Stryer, 1995) and the small subunit is highly conserved (Medina et al., 2001), this region has been the target region of

choice for the development of molecular assays. The present study carried out to isolate, and identify the dominant LAB strains from the kimchi samples in the initial and/or the middle stages of fermentation. Isolates were identified biochemically and at the molecular level using ITS- and RAPD-PCR and 16S rRNA gene sequence analysis.

Results

1. Classical identification of the kimchi isolates

A total of 20 isolates were randomly selected from agar plates of a range of selective media for phenotypic and biochemical characterization as described in Materials and Methods. At first, the major bacterial flora present in kimchi during the manufacturing process was detected by plating on the modified MRS media containing 10%(v/v) cabbage juice. All 20 isolates were considered LAB based on their positive Gram reactions, nonmotility, absence of catalase activity and spore formation (data not shown), and the strains were named YSM 1 to 20. Fifteen of the isolates produced gas from glucose, indicating a heterofermentative metabolism (Table 1, data not shown). Six of the isolates grew at both 10 and 45℃ after incubation for 5 days and 48 h, respectively. Twelve of the isolates were mesophilic and grew at 10℃ but not at 45℃. Two of the isolates grew well only at 45℃. The low counts of *Leuconostocs* increased slowly during manufacture but *Pediococci* remained very low in the early and middle fermenting stage of kimchi.

2. Culture-independent identification

ITS-PCR patterns: ITS-PCR is used for bacterial typing based on the variable between 16s and 23s rRNA with species. As for some reference strains of *Lb. brevis*, *P. acidilactici*, *P. pentosaceus*, *Leu. mesenteroides*, *Lc. lactis* subsp. *lactis*, ITS-PCR experiment were performed. The results of ITS-PCR fingerprinting showed that there were similar banding patterns within the same species and 450 and 700 bp for *Lb. plantarum*, 430-bp and 650-bp for *Lb. brevis*, 500-bp and 600 bp for both *P. pentosaceus* and *P. acidilactici*. *Leu. mesenteroides* produced one single band of 550-600 bp in size but 500-bp for *Lc. lactis* subsp. *lactis*. For the twenty isolates, YSM 1, 5, 7, 8, 10, 11, 12, 13, and 20 (Group 1) were nicely assigned to *Lb. plantarum* or *Lb. brevis*, YSM 2, 4, 6, 14, 17, and 18 (Group 2) were *Leu. mesenteroides* or *Lc. lactis* subsp. *lactis*. The banding pattern of YSM 9 (Group 3) was very close to the *Pediococcus* genus. However, no banding patterns of the reference strains were similar to those of YSM 3, 15, 16, and 19 (Group 4). Therefore, there needed to digest *Rsa*I, an endonuclease, the ITS-PCR amplicons for more accurate identification, As shown in Fig. 1, YSM 1 in the group 1 was assigned to *Lb. plantarum* and 4, 14, 18 in the Group 2 presumably belongs to *Leu. mesenteroides,* based on the banding patterns of the digested amplicons.

RAPD-PCR: RAPD was used to type 21 strains from fermenting kimchi (Cocconeiil et al., 1995). With primers ED-01 and ED-02, the RAPD patterns were shown in Fig 2, and compared them with those of the reference strains of lactic acid bacteria, resulting that there were 5 to 15 bands ranging from 150 to 1500-bp. When compared to the patterns of reference strains, the banding patterns of the

isolates had little similarities to the reference strains but similar patterns were observed for the strains of YSM 7, YSM 11, and YSM 12; YSM 14 and YSM 18. However, as the banding patterns were not corresponded to both the size and the number of bands between the strains tested, suggesting that the RAPD-PCR method was not feasible for typing the LAB isolates from fermenting kimchi.

16s rRNA sequence analysis: To our knowledge, 16s rRNA sequence analysis was known as the best typing method of bacterial classification no matter what it takes time and cost (Yoon, 2000). Genomic DNA of each isolate was purified. After performing 16s rDNA PCR, resulting 300-350 -bp products were sequenced at the Sequencing Center of University of California (Davis, CA) and compared its homology with Genebank database. 18 strains except for 2 strains had above 95% homology to the database, assigning the strains to 7 strains of *Lb. brevis*, 3 strains of *Weisella kimchii*, 2 strains of *Leu. medenteroides*, 2 strains of *Leu. citreum*, a single strain of *Leu. carnosum*, *Leu. gasicomitatum*, *W. hanii*, *Lb. plantarum*, *Lb. farciminis*. It is noteworthy that strains belong to *Weisella* genus along with *Lactobacillus* and *Leuconostoc* genus was prevalent. Contrary to our anticipation on major LAB flora in fermentation of kimchi, *Lactobacillus brevis* is apparently the most dominant species in the initial and/or the middle stage (pH 4.0 above) of fermenting kimchi,

Conclusion

To explore the major lactic acid bacteria in the initial and/or the middle stage of manufacturing of kimchi, traditional fermented food in Korea. Twenty one kimchi isolates, which were the most commonly recovered from the kimchi samples of pH 4.0 above, were selected for identification by using both a conventional biochemical identification method based on carbohydrate utilization and few culture-independent typing methods, including ITS-PCR, RAPD-PCR, and 16s rRNA gene sequence analysis. And then the results from the respective identification method tested were compared. The results have shown that biochemical test (API CHL kit) coincided with those of 16s rDNA sequencing data by about 60% at genus-level and about 40% at species-level. By contrast, ITS-PCR method coincided with those of 16s rDNA sequencing data by about 55% at genus-level and about 40% at species-level. It was also strongly suggested that RAPD-PCR method was not feasible for identifying lactic acid bacteria mainly because the resulting RAPD-PCR patterns of some reference strains on polyacrylamide gel didn't share those of kimchi isolates. Given 16s rDNA sequencing is the most reliable typing method used, the species distributions of twenty one dominant strains during kimchi fermentation at the initial and/or the middle stage was identified as follows: 7 strains of *Lactobacillus brevis*, 3 strains of *Weisella kimchii*, 2 strains of *Leuconostoc mesenteroides* and *Leu. citreum*, and a single strain of *Leu. carnosum, W. hanii, Leu. gasicomitatum, Lb. farciminis,* and *Lb. plantarum,* respectively. In conclusion, the present data suggest that the predominant LAB species in the initial and/or the middle stage of fermenting kimchi was not *Leu. mesenteroides*, in contradiction to a common hypothesis believed in a bacterial succession of kimchi fermentation, but *Lb. brevis*, a heterolactic fermenter.

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