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Research paper

Impact of clay minerals on bacterial diversity during the fermentation process of kimchi

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ABSTRACT

Kimchi is a popular traditional Korean food and has various beneficial health properties, e.g., it has preventative effects against cancer, obesity, diabetes, and constipation. The characteristics of kimchi, including its texture, flavor, and functionality, are affected by the major and minor ingredients. Bentonite, a clay mineral, is used as a component in functional foods, cosmetics, and pharmaceuticals owing to its physical and chemical characteristics. In this study, different types of cation-substituted forms of bentonite (Na^+, K^+ and Mg^{2+}) were used as kimchi ingredients to analyze their effect on the microbial community during fermentation for 10 days. Amplicon sequencing targeting the bacterial 16S rRNA gene was conducted using the ion torrent PGM system and sequencing data were analyzed with Qiime. At the initial stage, kimchi samples exhibited diverse microflora. Lactic acid bacteria (LAB), such as *Weissella, Lactobacillus*, and *Lactococcus*, dominated after 10 days of fermentation. Seven core LAB species, i.e., *Leuconostoc gelidum*, *Leuconostoc gasicomitatum*, *Weissella koreensis*, *Leuconostoc citreum*, *Leuconostoc lactis*, *Lactobacillus sakei*, and *Weissella cibaria*, showed differences in abundance among samples treated with different types of bentonite.

1. Introduction

Kimchi, a traditional food, has been consumed as a staple side dish since the third century AD in Korea (Chang, 1975). Approximately 200 varieties of Kimchi are currently available; these differ according to the type of main and minor ingredients, based on the local specialty and harvest season (Jang et al., 2015). Kimchi provides minerals, vitamins, proteins, and fibers and accordingly is a nutritional supplement for Koreans who consume steamed rice as a main dish (Cheigh et al., 1994).

Kimchi is normally manufactured by the following process: 1) various vegetables are soaked in brine and rinsed to get rid of saltiness, 2) minor ingredients, including red pepper powder, Jeotgal (salted seafood), mashed garlic, and ginger, as well as other tasty materials are prepared to make a sauce, and 3) ingredients are mixed together for fermentation (Kim, 2013). At the early stage of fermentation, diverse bacteria, such as *Proteobacteria*, *Enterobacteria*, *Weissella*, *Lactobacillus*, and *Leuconostoc*, are present, and specific LAB (lactic acid bacteria) increase as fermentation proceeds (Jung et al., 2012). Microorganisms in LAB, such as *Lactobacillus, Leuconostoc*, and *Weissella*, play an important role in the kimchi fermentation process (Jung et al., 2011) and have functional benefits (Park et al., 2014).

In 2012, kimchi was identified as one of the world's healthiest foods, along with olive oil, yogurt, soybean, and lentils (Bautista, 2012). Beneficial health effects, such as anti-oxidative potency against cellular oxidative stress (Lee et al., 2005b), immune-stimulatory activity of Peyer's patch cells (Lee et al., 2006), and weight reduction (Kwon et al., 2004), arise during the fermentation process. In addition, it contributes to the prevention of obesity, diabetes (Park et al., 2012b), constipation (Park et al., 2014), mutagenic effects, and cancer (Park, 1995). And certain type of kimchi, Dolsan leaf mustard Kimchi has beneficial health functions, such as anti-hypertensive, anti-oxidation, and detoxification functions (Yu, 2004).

Recently, efforts have been made to improve the quality, taste, and even functional properties of kimchi. Kimchi characteristics are affected by the addition of different minor ingredients, such as green onion, garlic, ginger, and red pepper (Yi et al., 1998). Additionally, green tea added to kimchi delayed the degree of kimchi textural changes and extended the storage period (Park et al., 1994). In addition, Kimchi

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supplemented with chitosan and chitosan-oligosaccharide showed antimutagenic functions (Kim et al., 2004).

Clay minerals affect the physical and chemical properties of materials owing to their porosity (Morris and Shepperd, 1982; Bergaya and Lagaly, 2006). Clay minerals can reduce aflatoxins in the animal stomach and intestines via absorption (Phillips et al., 2008) and improve wound healing as an antibiotic agent (Ghadiri et al., 2014). Bentonite is a clay mineral mostly comprised of smectite, in which the predominant composition of smectite in the bentonite is montmorillonite (Mt) (Kaufhold et al., 2002). It also has been applied to the development of health-related products, such as functional foods, cosmetics, and pharmaceuticals (Allo and Murray, 2004; Emami-Razavi et al., 2006). It has been used for the treatment of human osteosarcoma (Haroun et al., 2009) and skin wound healing (Emami-Razavi et al., 2006) as remedial agent, and manufacturing reservoirs having improved anticancer drug delivery proficiency (Kevadiya et al., 2012). For example, bentonite can effectively improve the symptoms of abdominal pain or discomfort in constipation-predominant irritable bowel syndrome (Ducrotté et al., 2005).

Based on previous studies, the growth of microorganisms was hypothesized to be affected by bentonite during the kimchi fermentation process. In this study, six types of bentonite (including bentonite modified with different cations, Na^+, K^+, and Mg^{2+}) were prepared by a cation exchange process and bentonite-treated kimchi products were manufactured. The bacterial compositions of these bentonite-supplemented kimchi samples were analyzed and compared using a next-generation sequencing (NGS) approach.

2. Materials and methods

2.1. Sample preparation & chemical properties

The bentonites used in this study were collected at the Gampo-35 and Gampo-46 mining area (denoted Bgp35 and Bgp46), respectively, located in Gyeongju, Republic of Korea (Fig. 1). These materials were purified by gravity separation methods for particle sizes of < 5 μm. The Na^+-, K^+- and Mg^{2+}-exchanged forms (Bgp35b.p.Na, Bgp35b.p.K, and Bgp35b.p.Mg, respectively) of Bgp35b.p, where b represents the blue color and p indicates purification, were prepared by stirring the Ca^{2+} form of Bgp35b.p in 0.5 M NaCl, KCl, and $MgCl_2$ solutions at room temperature for 18 h. The Bgp46b.p samples were similarly prepared. Powder X-ray diffraction (XRD) patterns were recorded on a Rigaku MiniFlex 600 diffractometer with Cu Kα radiation (λ = 1.5406 Å) at 40 kV and 15 mA. Data were collected over the 2θ range of 2° to 70° with a step size of 0.02°. Indexing of the XRD patterns obtained was carried out using the Crystallographica Search-Match (CSM) software (Oxford Cryosystems, Oxford, UK) (Figs. 2 and 3). In addition, Kunipia, commercial bentonite product manufactured in Japan, was used to

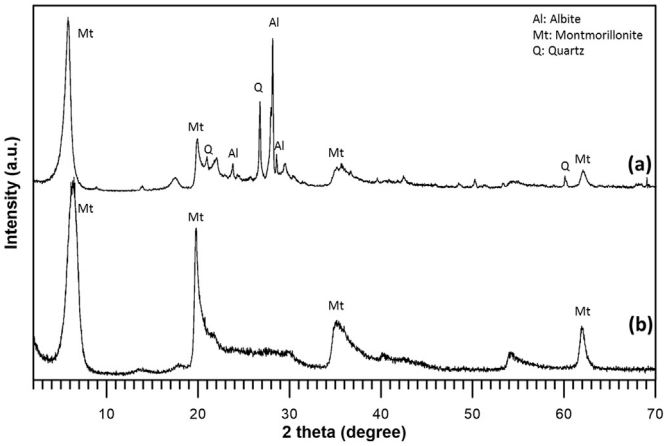

Fig. 2. XRD patterns of (a) the Gampo-35 bentonite and (b) purified bentonite.

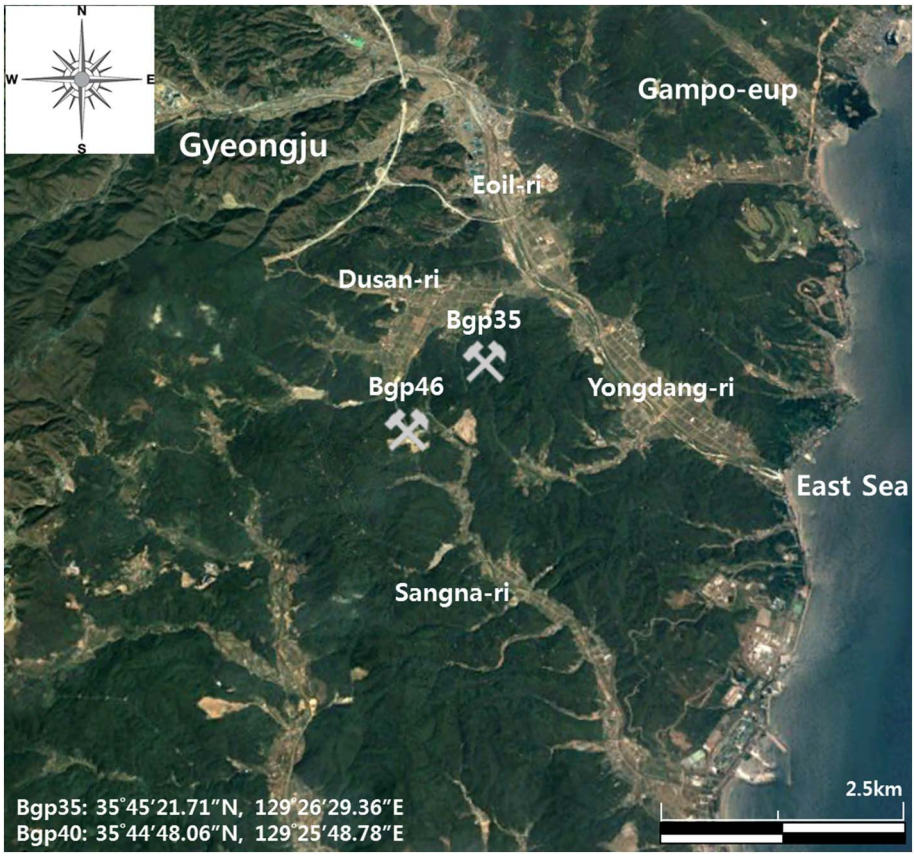

Fig. 1. Sampling site information.