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Full Length Research Paper

Phytate degradation by *Leuconostoc mesenteroides* KC51 cultivation in soymilk

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The phytate-degrading activity of *Leuconostoc mesenteroides* KC51 isolated from *Kimchi* was evaluated. When the phytase activity was measured in cultured broth on Lactobacilli MRS medium, the activity was detected in harvested cell suspension but not in the extracellular medium. The optimum pH was determined to be pH 5.5. *L. mesenteroides* KC51 cultivation in autoclaved soymilk resulted in a significant reduction of phytate content. After 9 h, around 47% of phytate had disappeared, and phytate content tended to stabilize around 50% of its initial value at the end of the 18 h fermentation. And decrease of phytate content was associated with *L. mesenteroides* KC51 cell growth during fermentation.

Key words: *Leuconostoc mesenteroides*, phytate degradation, soymilk.

INTRODUCTION

Phytate (*myo*-inositol hexakisphosphate) is the major phosphorus compound in plants and is particularly abundant in legumes, cereals, and oil seeds (Reddy et al., 1982). Phytate acts as an antinutritional factor for humans and monogastric animals because it may chelate nutritionally important cations such as Ca^{2+}, Mg^{2+}, Fe^{2+}, and Zn^{2+}, and basic amino groups in the proteins at physiological pH values, thus decreasing the dietary bioavailability of these nutrients (Wodzinski and Ullah, 1996). Specially, iron deficiency was reported as a conesquence of high phytate intakes (Hallberg et al., 1987). Therefore, excess phytate should be eliminated by suitable processing efforts.

Phytase catalyzes the hydrolysis of phytate into *myo*-inositol and phosphate and *via* penta- to monophosphates, thus decreasing or eliminating its antinutritional effect (Konietzny and Greiner, 2002). Phytases are used as feed additive to improve phosphate bioavailability and reduce the loss of divalent cations. Addition of phytase to food is also considered as a method to enhance the bioavailability of essential dietary minerals.

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Phytase is widely distributed in nature, occurring in plants, animal tissues, and microorganisms. Microbial sources of phytase are the most important ones for the production of this enzyme on a commercial level (Pandey et al., 2001). Particularly yeasts, lactic acid bacteria, and bifibobacteria are noteworthy phytase sources due to their various applications and safety (De Angelis et al., 2003; Oh and Lee, 2007; In et al., 2008).

Soybean-based products are widely consumed in many countries, because soybeans contain rich proteins, lipids, carbohydrate, minerals, and vitamins. In particular, soymilk or soymilk fermented with lactic acid bacteria and bifidobacteria may be a distinctive functional food because of growth stimulating factors such as oligosac-charides, amino acid, and peptides. However, the presence of significant amounts of phytate in these products can interfere with mineral absorption and lead to mineral deficiencies. Lactic acid fermentation was known to notably reduce the phytate content in plant-based foods; however, it has been most intensively studied in whole wheat bread making (Lopez et al., 2000; Reale et al., 2007; Palacios et al., 2008). Despite raw soymilk generally contains 0.1-0.4% phytate (Ishiguro et al., 2003), the published works about fermented soymilk with lactic acid bacteria were concerned with bacterial growth (Wang et al., 2002; Farnworth et al., 2007), but not with reduction of phytate level.

We previously reported the potential of *Leuconostoc mesenteroides* KC51 isolated from *Kimchi*, one of Korean traditional fermented foods, to produce the fermented soymilk (Oh and In, 2008). The aim of this work was to evaluate the phytate-degrading activity of *L. mesenteroides* KC51 and to confirm the reduction of phytate level during soymilk fermentation.

MATERIALS AND METHODS

Microorganism and media

The *L. mesenteroides* KC51 isolated from *Kimchi* (Oh and In, 2008) was used as starter microorganism. Stock culture was main-tained on Lactobacilli MRS agar (Difco Lab., Detroit, MI) slant and subcultured monthly. Starter culture was grown in Lactobacilli MRS broth (Difco Lab., Detroit, MI) at 30℃ for 15 h on a rotary shaker at 150 rpm.

Preparation and fermentation of soymilk

Soybeans were washed and soaked overnight in distilled water. After heating the soaked soybeans at 100℃ for 30 min, the soybeans were blended with 10 times their dry weight of distilled water for 3 min. The blended soybean slurry was filtered through a double-layered cheese cloth to obtain soymilk. Soymilk was dispensed and autoclaved at 121℃ for 15 min. The 50 ml of autoclaved soymilk was inoculated with 2.5 ml of starter culture. The soymilk containing *L. mesenteroides* was incubated statically at 30℃ for 18 h.

Assay of phytase activity

The cells were harvested by centrifugation and washed with 0.1 M acetate buffer (pH 5.5), and then the cell pellets were resuspended in 0.2 M acetate buffer (pH 4.5~6.5) for measurement of intracellular phytase activity. The enzymatic reactions were initiated by incubation 0.1 ml cell suspension with 0.9 ml of 2 mM sodium phytate in 0.2 M acetate buffer (pH 4.5 - 6.5). After incubation at 37℃ for 1 h, the reaction was stopped by adding 1.0 ml 10% trichloroacetic acid. The liberated inorganic phosphate was measured according to the ammonium molybdate method (Heinonen and Lahti, 1981). One unit (U) of phytase activity was defined as that which liberated one micro mole of phosphate per hour under the assay conditions.

Determination of phytate concentration

The phytate concentration of the fermented suspension was measured by the Wade method (Latta and Eskin, 1980). The sample of fermented soymilk was centrifuged at 3,000 x g for 10 min. The supernatant (0.3 ml) was added to 2.7 ml DW and 1.0 ml of Wade reagent (0.03% $FeCl_3 \cdot 6H_2O$ and 0.3% sulfosalicylic acid in DW). The mixture was stirred on a vortex mixer and then filtered with 0.45 µm syringe filter; the absorbance of the filtrate was read at 500 nm. Concentration of phytate was shown mean values and standard deviation (n=3).

RESULTS AND DISCUSSION

To evaluate the phytate breakdown activity of lactic acid bacteria, *L. mesenteroides* KC51 strain was assayed for aerobic halo formation after 48 h incubation at 30℃ on MRS agar plate containing 0.5% calcium phytate. As a clear zone is appeared around colony (data not shown), it was estimated that the *L. mesenteroides* KC51 strain was able to degrade the phytate. Secondly, phytase acti-vity was measured in cultured broth on Lactobacilli MRS medium. This activity was detected in harvested cell suspension but not in the extracellular medium in agreement with previous works (De Angelis et al., 2003; Palacios et al., 2008). The effect of pH on enzyme activity was examined in 0.2 M acetate buffer (pH 4.5~6.5). The optimum pH was determined to be pH 5.5, with a sharp decline in activity as the pH moved toward the neutral range (Figure 1). The most of lactic acid bacteria showing the phytase activity were reported as *Lactobacillus* sp. isolated from sourdoughs (De Angelis et al., 2003; Palacios et al., 2008). Reports on the presence of *Leuconostoc* sp. having the activity of phytate degradation were extremely scarce. Despite of recent report that high level of phytate degradation was measured in one strain of *L. mesenteroides* from Cornetto di Matera sourdoughs (Zotta et al. 2007), the optimal pH conditions for phytase of *L. mesenteroides* strain was measured for the first time.

The extent of phytate degradation during lactic acid fermentation in soymilk was determined. *L. mesenteroides* KC51 cultivation in soymilk resulted in a significant reduction of phytate content (Figure 2). After 9 h, around 47% of phytate had disappeared, and phytate content tended to stabilize around 50% of its initial value at the end of the 18 h fermentation. Because the endogenous soybean phytase was inactivated by autoclaving at 121℃ for 15 min such as inactivation of intrinsic cereal phytase (Reale et al., 2007), the phytate content decreased as a consequence of the activity the *L. mesenteroides* KC51 phytase during fermentation. Around 50% breakdown of initial content was similar with recent research indicating that phytate in soybean-curd whey was degraded to 40-60% by *Saccharomyces cerevisiae* CY phytase (In et al., 2007, 2008). Possible reasons why the phytate reduction was not complete was that the phytase activity of *L. mesenteroides* KC51 was relatively low such as that of other lactic acid bacteria (Reale et al., 2007) and that the optimal pH lasted only for a short period, as the fermentation was performed at 30℃. In the previous report (Oh and In, 2008), it was confirmed that pH of fermented soymilk decreased below 4.5 after 12 h fermentation with *L. mesenteroides* KC51 strain. And then, decrease of phytate content during *L. mesenteroides* KC51 cultivation was concomitant with increase in the viable cell population within 18 h. This result might be seemed that phytase of *L. mesenteroides* KC51 was intracellular enzyme and acted as cell growth-associated type. This suggestion was supported by the finding that phytase activity was not detected in the extracellular MRS medium in this study and most phytases of lactic acid

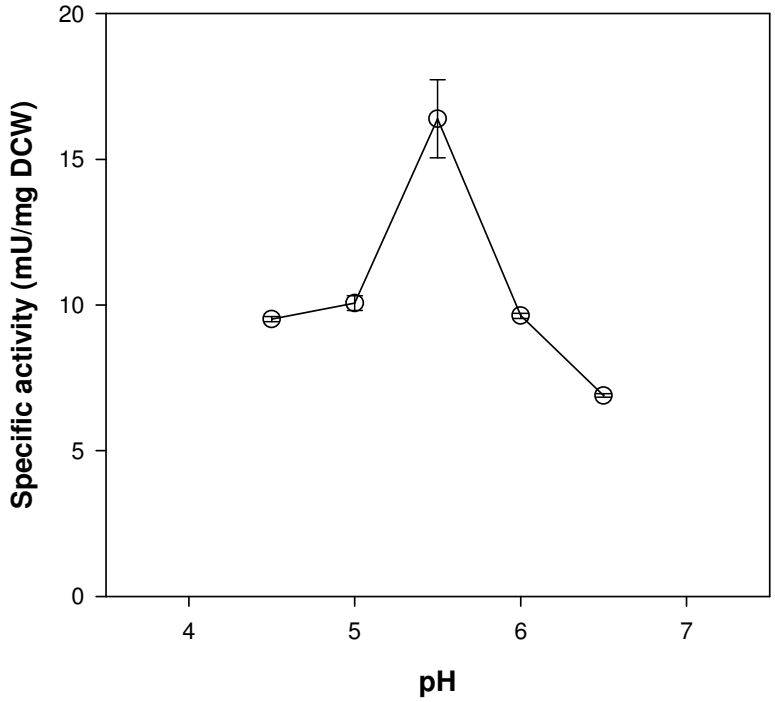

Figure 1. Effect of pH on the activity of the intracellular phytase from *L. mesenteroides* KC51 strain.

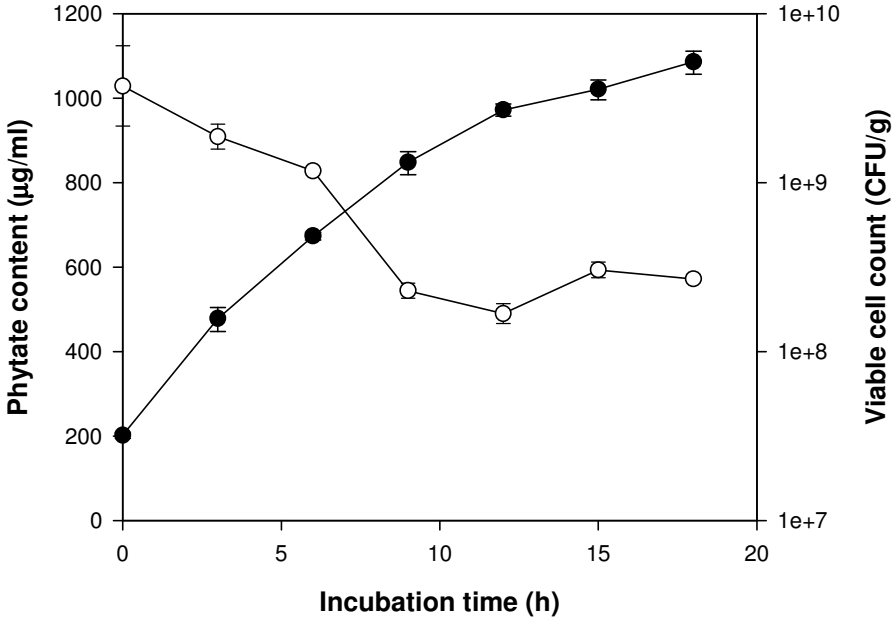

Figure 2. Change of phytate content (○) and growth of *L. mesenteroides* KC51 (●) in fermented soymilk during lactic acid fermentation at 30℃. The cell growth data were taken from Oh and In, (2008).

bacteria were generally assayed using harvested cells (De Angelis et al., 2003; Zotta et al. 2007; Palacios et al., 2008). From above results, it was clearly demonstrated that *L. mesenteroides* KC51 strain produced intracellular phytase and its cultivation significantly reduced phytate content in soybean-based foods.

REFERENCES

De Angelis M, Gallo G, Corbo MR, McSweenet PLH, Faccia M, Giovine M, Gobbetti M (2003). Phytase activity in sourdough lactic acid bacteria: purification and characterization of a phytase from *Lactobacillus sanfrancisensis* CB1. Int. J. Food Microbiol., 87: 259-270.

Farnworth ER, Mainville I, Desjardins MP, Gardner N, Fliss I, Champagne C (2007). Growth of probiotic bacteria and bifidobacteria in a soy yogurt formulation. Int. J. Food Microbiol., 116: 174-181.

Hallberg L, Rossander L, Skanberg AB (1987). Phytates and the inhibitory effect of bran on iron absorption in man. Am. J. Clin. Nutr., 45: 988-996.

Heinonen JK, Lahti RJ (1981). A new and convenient colorimetric determination of inorganic orthophosphate and its application to the assay of inorganic phoaphatase. Anal. Biochem. 113: 313-317.

In MJ, Kim KH, Oh NS (2007). Phytate degradation by immobilized *Saccharomyces cerevisae* phytase in soybean-curd whey. Biotechnol. Bioprocess Eng. 12: 348-353.

In MJ, Seo SW, Oh NS (2008). Fermentative production and application of acid phytase by *Saccharomyces cerevisiae* CY strain. Afr. J. Biotechnol. 7: 3115-3120.

Ishiguro T, Ono T, Nakasato K, Tsukamoto C, Shimada S (2003). Rapid measurement of phytate in raw soymilk by mid-infrared spectroscopy. Biosci. Biotechnol. Biochem, 67: 752-757.

Konietzny U, Greiner R (2002). Molecular and catalytic properties of phytate-degrading enzymes (phytase). Int. J. Food Sci. Technol. 37: 791-812.

Latta M, Eskin M (1980). A simple and rapid colorimetric method for phytate determination. J. Agric. Food Chem. 28: 1313-1315.

Lopez HW, Ouvry A, Bervas E, Guy C, Messager A, Demigne C, Remesy C (2000). Strains of lactic acid bacteria isolated from sour doughs degrade phytic acid and improve calcium and magnesium solubility from whole wheat flour. J. Agric. Food Chem. 48: 2281-2285.

Oh NS, In MJ (2008). Production of a fermented soymilk using a new strain *Leuconostoc mesenteroides* KC51 isolated from *Kimchi*. J. Korean Soc. Appl. Biol. Chem. 51: 88-91.

Oh NS, Lee BH (2007). Phytase properties from *Bifidobacterium animalis*. Food Sci. Biotechnol., 16: 580-583.

Palacios MC, Haros M, Sanz Y, Rosell CM (2008). Selection of lactic acid bacteria with high phytate degrading activity for application in whole wheat breadmaking. LWT-Food Sci. Technol. 41: 82-92.

Pandey A, Szakacs G, Soccol CR, Rodriguez-Leon JA, Soccol VT (2001). Production, purification and properties of microbial phytases. Bioresour. Technol. 77: 203-214.

Reale A, Konietzny U, Coppola R, Sorrentino E, Greiner R (2007). The importance of lactic acid bacteria for phytate degradation during cereal dough fermentation. J. Agric. Food Chem. 55: 2993-2997.

Reddy NR, Sathe SK, Salunkhe DK (1982). Phytates in legumes and cereals. Adv. Food Res. 28: 1-92.

Wang YC, Yu RC, Chou CC (2002). Growth and survival of bifidobacteria and lactic acid bacteria during the fermentation and storage of cultured soymilk drinks. Food Microbiol. 19: 501-508.

Wodzinski RJ, Ullah AHJ (1996). Phytase. Adv. Appl. Microbiol. 42: 263-302.

Zotta T, Ricciardi A, Parenta E (2007). Enzymatic activities of lactic acid bacteria isolated from Cornetto di Matera sourdoughs. Int. J. Food Microbiol., 115: 165-172.