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The combined impact of sauerkraut with Leuconostoc mesenteroides to enhance immunomodulatory activity in Escherichia coli-infected mice

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Abstract

This study investigated the pooled impacts of sauerkraut and *Leuconoctoc mesenteroides* culture on immunomodulatory activity in experimental animal. The in vivo immunomodulatory activity of *Escherichia coli* infected-Balb-C mice was ascertained in fermented sauerkrauts [test vs. control]. Both sauerkrauts enhanced the adaptive immune-response [evidenced by an increase in $CD4^+$ $CD8^+$ IFN-γ, TNFα] and innate immune response [represented by a decrease of CD68- IL-6]. Nevertheless, the in vivo

immunomodulatory activity of sauerkraut combined with *Leconoctoc mesenteroides* was higher than that showed in sauerkraut control solely.

Keywords: immunomodulatory activity; sauerkraut; *Leuconostoc mesenteroides*; mice

Introduction

The immune system plays a pivotal role in maintaining the body-integrity against foreign objects and pathogens. Bacterial infection poses negative impact on immune system by reducing its capacity and may cause disease. Immunomodulator is defined as compound that enhances the immune system capacity [1-2]. Sauerkraut has been reported as effective, potent immunomodulatory. Sauerkraut is a cabbage vegetable produced by the fermentation of lactic acid bacteria (LAB) which occurs spontaneously with the addition of salt. *Leuconostoc mesenteroides* is a heterofermentative Gram positive bacterium that plays key roles in fermentation of foods such as: kimchi, sauerkraut, and milk, leading to the production of various organic acids and aromatic compounds. Additional, bacteria species that have role in fermentation process are: *Leuconostoc mesenteroides*, *Lactobacillus cucumeris*, *Lactobacillus plantarum* and *Lactobacillus pentoacetius* [3-4].

At the beginning of fermentation process, *Leuconostoc mesenteroides* dominate to produce lactic and acetic acids that decrease pH. The fermentation process is then sustained by the bacteria *Lactobacillus plantarum* and *Lactobacillus brevis* until the pH reaches 3 [5-6]. The addition of *L. mesenteroides and L. plantarum* cultures accelerate the fermentation process and reduce the amount of added salt [7-8].

In the literature it was noted that lactic acid bacteria increases vitamins, phenolic and glucosinolate compounds of Sauerkraut [insert reference, please]. Meanwhile, phenolic compounds are famous with their antioxidant activity and the ability to scavenge free radicals, [10-11]. Sulforaphan which is an isothiocyanate derivative, has the ability to prevent cancer through DNA protection by modulating enzymes and inhibiting gene mutations [9-13]. Notwithstanding, a study on the addition of *Leuconostoc mesenteroides* as immunomodulator has never been reported, hence, this study aimed to investigate the immunomodulatory activity of sauerkraut combined with *L. mesenteroides* culture.

Materials and Methods

Materials

White cabbage (*Brassica olerace* L. var) were obtained from local markets. *Leuconostoc mesenteroides* [FNCC 0023] was obtained from Food and Nutrition Culture Collection [give details]. All chemical used were analytical grade purchased from local distributors.

Sauerkraut Production

Fresh cabbage was washed, shredded, before the addition of salt at a concentration of 0.5%, then inoculated with 20% culture [give details is it *Leuconostoc mesenteroides* ?], and incubated at room temperature (28^{o}C) for 5 days. A control sauerkraut was prepared with salt concentration at 2% without culture addition. Subsequently, the prepared sauerkrauts were subjected to quality analysis and immunomodulatory activity assay.

Sauerkraut quality analysis

Total lactic acid bacteria was determined according to Penas et al. [9] with counting the colonies grow on MRS Agar after incubation at 37^{o}C for 48 hours. Titratable acidity was measured according to Rangana [13] using direct titration with NaOH solution of 0.1N and expressed as % lactic acid. pH was measured by using pH meter (Manual pH meter Micro Bench TI 2100). Total phenolic content was determined according to Yang *et al*. [14] with measurement of complex compound formed, after the reaction with Folin Ciocalteau reagent and Na_2CO_3 solution, spectrophotometrically at 750 nm, and the content was expressed as mg GAE/g. DPPH scavenging activity was determined by measuring the absorbance at 517 nm, and was expressed as IC_{50}. Sulforaphane content analysis was carried out using *Liquid Chromatography-Mass Spectrometry* according to Kim *et al*. [15] under the below conditions:

HPLC system was equipped with API 400 Q TRAP mass spectrometry system, electrospray ionization mass (ESI) on positive ions ([M + H] +) mode, ion spray voltage (5.5 kV), gas (20 psi), nebulisation gas (50 psi), heater gas (50 psi), nitrogen purity (N2), heater gas temperature (550^{o}C), de-clustering potential (100 V), entrance potential (10 V), and spectrum range (m/z 100-1000) in 4.8 seconds.

Immunomodulatory activity assay

The immunomodulatory activity assays of the sauerkrauts were performed in vivo with 20 female six-week-old Balb/c mice, 18-20 grams weight. The experimental protocols and procedures of care and use of animals used in the present work were approved (ethical clearance No. KEP-751-UB) by the Ethics Committee. The National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) was followed in the this experiment. After 7 days adaptation, mice were divided into

four groups: P0 (negative control), P1 (positive control), P2 (sauerkraut without culture) and P3 (sauerkraut with culture). Sauerkrauts were administered orally at a dose of 0.15ml / kg / BW / day for 14 days. *E. coli* of $1.3x10^8$ CFU / ml was injected into the mice on the 15^{th} day and then incubated for 5 days, and then $CD4^+$ $CD8^+$ INF-□, TNF-α^+ and $CD68^+$ IL-6 were analyzed with flowcytometry. Total *E. coli* in intraperitoneal fluid was determined on violet red bile agar (VRBA) with incubation at 37°C for 24 hours [16].

Statistical analysis

Data were analyzed by complete random design along with analysis of variance (ANOVA) and further analysis by Tukey at α=5%.

Results and Discussion

Sauerkraut quality

During the sauerkraut fermentation, lactic acid bacteria grew and created the sauerkraut characteristics. Table 1 presents the results of the sauerkraut quality analysis.

Table 1 Sauerkraut quality with and without *L. mesenteroides* culture addition

Parameter	Sauerkraut with culture	Sauerkraut without culture
Lactic acid bacteria (CFU/ml)	2.40×10^8	2.60×10^7
Titratable acidity (%)	1.41 ± 0.01	0.80 ± 0.02
pH	3.67 ± 0.06	4.97 ± 0.09
Total Phenolic content (mg GAE/g)	72.24 ± 0.92	46.59 ± 0.42
Antioxidant activity (IC_{50}, ppm)	95.39 ± 2.37	135.12 ± 2.75
Sulforaphane content (ng/g)	848.65	776.47

Total LAB in sauerkraut with culture addition was higher than that of control, which resulted in higher titratable acidity and lower pH value. This can be explained on the basis that lactic acid bacteria synthesize various enzymes such as invertase, cellulase, and amylase which are capable of breaking the complex between phenol compounds and tissue or cell structures to release the phenolic compounds [17-18]. Lee *et al*. also reported that fermentation of mulberry leaves by *L. plantarum* increase the total phenol, due to duration of the fermentation [19]. These activities resulted an increasing in total phenol and DPPH

scavenging activity. Data of sulforaphane content reflected that during fermentation the lactic acid bacteria produce myrosinase, which is capable of transforming glucoraphanin into sulforaphane compounds.

Immunomodulatory activity of the sauerkraut

The immune response analysis in this study was carried out on the T cell adaptive immune response with cytokines $CD4^+$, $CD8^+$, IFN-γ^+, TNF-α^+. The results are presented in Table 2.

Table 2 Immunomodulatory activity of sauerkraut

Group	$CD4^+$IFN γ^+ (%)	$CD4^+$TNF α^+ (%)	$CD8^+$IFN γ^+ (%)	$CD8^+$TNF α^+ (%)
Control negative (P0)	0.36 ± 0.14^{c}	0.44 ± 0.19^{b}	0.23 ± 0.04^{c}	1.57 ± 0.14^{c}
Control positive (P1)	0.51 ± 0.11^{c}	0.78 ± 0.30^{b}	0.43 ± 0.17^{bc}	5.08 ± 1.01^{a}
Sauerkraut without culture (P2)	1.50 ± 0.27^{b}	1.17 ± 0.38^{ab}	0.64 ± 0.14^{b}	3.02 ± 0.17^{b}
Sauerkraut + culture (P3)	2.07 ± 0.67^{a}	1.98 ± 1.30^{a}	1.30 ± 0.20^{a}	2.28 ± 0.54^{c}

Note: Values are means ± standard deviations (n=5). Different letter in the same column mean significant different at $\alpha=5\%(p < 0.05)$.

The results of cytokines in spleen were significantly different ($p<0.05$) between the sauerkraut with and without *L. mesenteroides* culture. It was reported that IFN-γ induces macrophages by improving their ability to kill bacteria and parasites, while TNF-α inhibits the replication of intracellular pathogenic bacteria and directly kill infected cells. Notably, $CD4^+$ functions as a co-receptor that strengthens the transduction signal so that T cells are activated, whereas $CD8^+$ is a transmembrane protein that functions as a co-receptor on killer T cells. Castillo *et al*. has reported that lactic acid bacteria in mice can increase TLR2, TLR4, and TLR9 expression and surge TNF-α, IFN-γ and IL-10 secretion in Peyer patche's [20].

The immune response analysis process was carried out on innate immune responses on CD68 and IL-6 macrophages (Figure 1). Statistical analysis results showed significant differences ($\alpha = 0.05$) between the sauerkraut without culture and that with culture. The reduction of $CD68^+$ $IL\text{-}6^+$ level is due to sauerkraut stimulation and enhancement of innate immune system when infected with *E. coli*, macrophage which can work against pathogens and phagocytosis and normalizing the infected immune system. Furthermore, lactic acid bacteria inhibits inflammation and activates the innate immune system that balances the Th1 and Th2 responses so that they can fight off pathogenic bacterial infections. Lactic acid bacteria also modulates the expression of cytokines, maturation of immune cell surface markers, and increases lymphocyte proliferation. IL-6 is a multifunctional cytokine that regulates immune responses, acute phase responses,

hematopoiesis, and inflammation. This release of IL-6 stimulates macrophage cells to maturation stage so they are able to carry out phagocytosis more efficiently [21-22].

Lactic acid bacteria in sauerkraut plays a pivotal role in phagocytic pathogens. Lactic acid bacteria inhibits the growth of microorganisms by decreasing the pH of the environment. Total *E. coli* decreased after the treatment with sauerkrauts (Table 3). Bioactive compounds and BAL in sauerkraut improve the performance of the immune and antibacterial response. Furthermore, 2-phenylethyl isothiocyanate is one of the bioactive substances present in cabbage with antimicrobial ability [23-24].

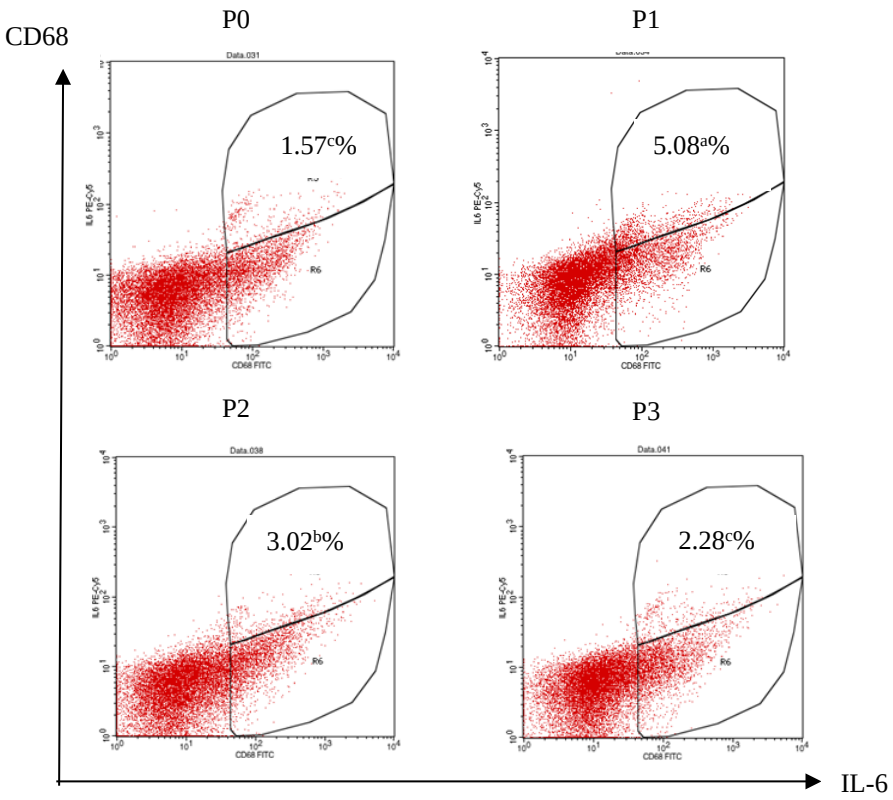

Fig. 1 $CD68^{+}IL\text{-}6^{+}$ macrophage cells at different treatments: (P0) control negative, (P1) control positive, (P2) sauerkraut without culture, (P3) sauerkraut + culture

Table 3 Total *E. coli* in mice at different treatments

Group	Total *E. coli* (CFU/ml)
Control negative (P0)	-
Control positive (P1)	2.3×10^{7}

Sauerkraut without culture (P2)	3.3×10^3
Sauerkraut with culture (P3)	1.7×10^2

Conclusion

Our findings highlighted that sauerkraut enhances the adaptive immune respone [evidenced by an increase in $CD4^+$ $CD8^+$ IFN-γ, TNFα] and innate immune response [denoted by a decrease of CD68- IL-6]. However, the in vivo immunomodulatory activity of sauerkraut combined with *Leconoctoc mesenteroides* was much higher than that showed in sauerkraut without fermenting bacteria.

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