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RESEARCH ARTICLE

Antibacterial Activities of Isothiocyanates (ITCs) Extracted from Horseradish (*Armoracia rusticana*) Root in Liquid and Vapor Phases against 5 Dominant Bacteria Isolated from Low-salt *Jeotgal*, a Korean Salted and Fermented Seafood

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Abstract Antibacterial activities of isothiocyanates (ITCs) extracted from horseradish (*Armoracia rusticana*) root in liquid and vapor phases were evaluated against 5 dominant bacteria (2 strains of *Bacillus* sp., *Straphylococcus* sp., *Streptococcus* sp., and *Enterobacter* sp.) from *jeotgal*. ITCs in liquid and vapor phases exhibited potent antibacterial activities based on a disc diffusion method (liquid phase) and a disc volatilization method (vapor phase). ITCs minimum bactericidal concentrations in the liquid phase (broth dilution method) and vapor phase (disc volatilization method) ranged from 0.208±0.091 to 2.083±0.722 mg/mL and 0.078±0.000 to 1.875±0.722 mg/mL, respectively. ITCs in the vapor phase showed stronger antibacterial activities against all tested bacterial strains. ITCs extracted from horseradish root in the vapor phase can be used as a natural preservative to extend the shelf-life of *jeotgal*.

Keywords: *jeotgal*, antibacterial activity, vapor phase, isothiocyanate (ITC), horseradish root

Introduction

Jeotgal or *jeot* is a traditional fermented food in Korean cuisine that is used as an important additive for improving the taste of foods, or as a stand-alone food. It is usually prepared using addition of salt to shrimp, oyster, shellfish, fish, roe, or fish intestines before fermentation (1-3).

In Korea, *jeotgal* is usually made using fermentation with highly salted (20-30%) marine animals. However, a high salt intake may be crucial for development of hypertension (4) and may increase the risk of stroke and cardiovascular disease for consumers (5). Thus, reduction of salt intake is recommended. This presents a challenge for the *jeotgal* industry to reduce salt content in the product. However, low-salt *jeotgal* (8-10%) has safety problems due to the possible presence of pathogens and a reduction in shelf-life because of a high level of microorganisms and an associated high water activity (Aw=0.95), compared with high-salt *jeotgal* with Aw=0.88 (2,6). A salt content of only 8-10% may not be sufficient for inhibition of pathogenic bacterial growth in *jeotgal* (7). Moreover, the opportunistic pathogenic bacteria *Enterococcus* sp., *Paenibacillus tyraminigenes,* and *Bacillus licheniformis* may be present in *jeotgal* (8-10). Low-salt *jeotgal* can also be subject to spoilage and quality deterioration, leading to a risk for consumers, and increased costs and marketing issues for *jeotgal* industries.

Modern preservation techniques, such as genetic engineering, irradiation of food, and modified-atmosphere packaging, are methods for control of spoilage bacteria (11). However, use of synthetic antimicrobial agents and chemical food preservatives is one of the oldest techniques that are still in use. However, due to growing evidence of harmful effects and health problems of chemicals, there is pressure to reduce the amounts of synthetic preservatives used in foods (12), increasing the need to find natural methods, such as use of essential oils, for reduction or elimination of food-

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related spoilage bacteria, resulting in an extension in product shelf-life (12).

Horseradish (*Armoracia rusticana*) of the Brassicaceae family contains allyl isothiocyanate (AITC, >65%), 2-phenylethyl isothiocyanate (PITC, >30%), and other isothiocyanates (ITCs) (13). ITCs showed antimicrobial activities against microorganisms, including fungi and bacteria. AITC has potential for inhibition of pathogenic microorganisms even at low concentrations (14,15). Moreover, use of AITC as a natural food preservative in real food systems has also been achieved with chicken breast, dry cured ham, fermented dry sausages, cottage cheese, fruit, and cooked rice (16-22).

Antimicrobial activities of essential oils have been widely studied in the liquid phase, usually using direct contact antimicrobial assays, such as diffusion or dilution methods. Although essential oils in the liquid phase have a high efficiency against food-borne and spoilage micro-organisms, the effect in food is only achieved at high concentrations (12,23). Thus, evaluation of essential oils as food preservatives in real food systems requires consideration of reactivity and sensory effects.

An alternative method for consideration of the sensory effect is use of the vapor phase of essential oils, which can be effective at lower concentrations than for the liquid phase due to high hydrophobicity and volatility, thereby causing a minimum sensory effect (24,25). Kloucek *et al*. (26) and Nedorostova *et al*. (24) studied antimicrobial activities in the vapor phase of many essential oils and developed an antimicrobial packaging. In addition, ITCs vapor extracted from horseradish showed a minimum inhibitory concentration of 8.3-31.25 μL/L of air in Petri dishes against food-borne microorganisms (24). However, few studies exist regarding antimicrobial activities of ITCs extracted from horseradish (*A. rusticana*) and comparison of results between the liquid and vapor phases.

Thus, the objectives of this study were to (1) to compare the antibacterial activities of ITCs extracted from horseradish in liquid and vapor phases against 5 dominant bacteria isolated from squid, *myeong-ran,* and *chang-ran jeotgal* varieties using antibacterial assays, and (2) to evaluate use of ITCs as natural food preservatives for extension of the *jeotgal* shelf-life.

Materials and Methods

Preparation of ITCs from horseradish root Powdered horseradish root (*A. rusticana*) from China was purchased from Biocoats Co., Ltd. (Seoul, Korea). ITCs were extracted using a steam distillation method (27) with 200 g of horseradish powder mixed with 550 mL of distilled water, followed by incubation at 40°C in a water bath for 2 h for maximum production of ITCs. The reacted mixture was distilled and concentrated at 120°C in an oil bath for 120 min by rotary evaporator (Rotavapor R-200; BÜCHI Labortechnik AG, Flawil, Switzerland). Essential oils (ITCs solution, precipitate) were separated from the concentrated solution using centrifugation (Bionova, Model Mega 17R; Hanil Science Industrial, Seoul, Korea) at 5,000×*g* for 20 min.

ITCs extracted from horseradish root were analyzed for identification of the main active components involved in antibacterial activities using a 7890A GC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a 5975C mass spectrometer (Agilent Technologies). ITCs were prepared using dissolution in *n*-hexane (Showa Chemical Industry Co., Tokyo, Japan) at a 1:1 ratio. The hexane fraction was separated using an HP-5 column (30 m×0.25 mm I.D., 0.25 μm film thickness; Agilent Technologies). The column temperature was held at 50°C for 1 min, then increased to 250°C for 3 min. The inlet temperature was 250°C for 3 min. Helium was used as a carrier gas at a flow rate of 1.0 mL/min. The extraction yield of ITCs was determined using standard concentrations of AITC (Wako, Tokyo, Japan), PITC (Sigma-Aldrich, St. Louis, MO, USA), and BITC (Sigma-Aldrich).

***Jeotgal* samples and dominant bacteria isolation** Low-salt (approximately 5% NaCl) squid, *myeong-ran,* and *chang-ran jeotgal* varieties were purchased from Jin-Il Manna Food Co., Ltd. (Sokcho, Korea) in November of 2012. Samples of each *jeotgal* (30 g) were aseptically transferred to sterile plastic filter bags (Nasco Arts & Crafts Store, Modesto, CA, USA) and homogenized thoroughly for 1.5 min in a stomacher (Stomacher 400 circulator; Seward Ltd., Worthing, UK) with 270 mL of 1.5% sterile peptone water (Difco, Franklin Lakes, NJ, USA) supplemented with a 5% sodium chloride solution (Samchun Chemical Co., Pyeongtaek, Korea) and incubated at 25°C for 24 h. Dilutions of *jeotgal* samples were prepared in saline. Aliquots of 0.1 mL of each dilution were spread onto brain heat infusion (BHI) agar (Difco) supplemented with 5% NaCl and incubated at 25°C for 72 h. Different types of colonies were collected from each plate based on differences in morphology and colony numbers. Collected colonies were purified based on streaking on BHI agar and incubated in aerobic incubator (BITEC-500; Shimadzu Co., Tokyo, Japan) at 25°C. Single colonies were selected and stored on BHI agar slant at 4°C for subsequent use.

Identification of isolates Biochemical testing using an automated microbiology system (MicroStation 62402; Biolog Inc., Hayward, CA, USA) was used to identify and characterize isolated dominant bacteria, Gram-staining,

shape, spore formation, motility, oxygen requirements, catalase/oxidase testing, formation of indole, and glucose/lactose fermentation were carried out following Bergey's Manual of Systematic Bacteriology (28).

Selection of dominant bacteria Colonies with high population numbers in *jeotgal* were selected based on isolation frequency and diversity. Six isolates were selected from the three kinds of *jeotgal* for use as dominant bacteria.

Inocula preparation Strains of isolated bacteria were grown in BHI broth with 5% NaCl and incubated at 25°C for 72 h. Cell suspensions were adjusted for turbidity using BHI broth with 5% NaCl to achieve a cell density of 1.0×10^8 CFU/mL (0.1-0.2 of optical density at 600 nm) by Mcfarland standard method (29).

Disc diffusion method The disc diffusion method (30) was used for determination of antibacterial activities of ITCs in the liquid phase. Cell suspensions of dominant bacteria (100 μL of 1.0×10^7 CFU/mL) were spread on BHI agar plate with 5% NaCl. Then, sterile filter paper discs of 6 mm diameter (Advantec, Tokyo, Japan) were fixed on the inoculated plates and 10 μL of 25 mg/mL ITCs were dropped onto the fixed sterile filter paper discs. The agar plates were incubated at 25°C for 72 h. Inhibition zones were measured as a ratio of the diameter of the clear zone to the diameter of the filter paper disc. Triplicate samples were prepared for each treatment.

Disc volatilization method The disc volatilization method (30) was used for determination of antibacterial activities of ITCs in the vapor phase. One hundred μL of each cell suspension containing 1.0×10^8 CFU/mL was spread on the surface of BHI agar plate with 5% NaCl. A paper disc (diameter 6 mm) was placed on the inside surface of the upper lid and 10 μL of 25 mg/mL ITCs was dropped on each disc. Plates inoculated with bacteria were immediately inverted and the lid and sealed using parafilm (Bemis Company, Neenah, WI, USA). The agar plates were incubated at 25°C for 72 h and the diameters of inhibition zones were measured as a ratio of the diameter of the clear zone to the diameter of the filter paper disc. Triplicate samples were prepared for each treatment.

Determination of minimum inhibitory concentrations and minimum bactericidal concentrations

***Broth dilution method*:** The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of ITCs were determined following the broth dilution method modified from the method of Devkatte *et al.* (31). All tests were performed in 2 mL micro-screw cap tubes containing BHI broth with 5% NaCl. Bacterial strains were cultured at 25°C for 72 h in BHI broth with 5% NaCl. ITCs were serially diluted in a two-fold series with sterilized BHI broth. Each micro-tube containing 850 μL of medium was inoculated with 100 μL of a cell suspension (final density 1.0×10^5 CFU/mL) of a bacterial strain and 50 μL of ITCs of each dilution. Final concentrations ranged from 0.078 to 10 mg/mL. The total volume in each micro-tube was 1,000 μL (850 μL of medium+100 μL of cell suspension +50 μL of ITCs). Triplicate samples were prepared for each treatment. Micro-tubes containing only BHI broth with 5% NaCl as a control were incubated at 25°C for 72 h. MIC values were measured based on visual inspection and determined as the lowest concentration of ITCs that prevented visible growth of microorganisms. MBC values were determined as the lowest concentration of ITCs at which no growth was observed after sub-culturing into fresh BHI agar plate with 5% NaCl incubated at 25°C for 72 h.

***Disc volatilization method*:** All test was performed using BHI agar plate with 5% NaCl. A 100 μL cell suspension was spread on fresh BHI agar plate with 5% NaCl at final density of 1.0×10^5 CFU/mL. A 5C disc paper with 3.5 cm diameter (Advantec) was placed on the inside surface of the upper lid. ITCs were serially diluted in a two-fold series with sterilized BHI broth, then ITCs were vigorously mixed by vortex and immediately dropped onto the sterile paper discs at final concentrations ranging between 0.020 to 2.500 mg of ITCs/mL in the headspace of a Petri dish calculated based on division of the ITCs concentrations (mg) by the Petri dish headspace volume (mL) (32). Plates inoculated with bacteria were immediately inverted and the lid and sealed using parafilm. Agar plates were incubated at 25°C for 72 h, the paper discs were then removed, followed by incubation again under the same conditions for 72 h. Triplicate samples were prepared for each treatment. Plates prepared without ITCs were used as a control. MIC values were defined as the lowest concentration of ITCs resulting in lack of visible growth of microorganisms on agar plate. MBC values were defined as the lowest concentration of ITCs showing lack of re-growth after ITCs were removed from agar plate and then were re-incubated.

Statistical analysis All experiments were performed in triplicate and data were recorded as mean values±standard deviation (SD). Ratio data for inhibition zones, and MIC and MBC values for ITCs were analyzed using a one-way analysis of variance (ANOVA) and Duncan multiple range test with SPSS (version 19; SPSS Inc., Chicago, IL, USA) statistical software. A level of significance of $p<0.05$ was used for all comparisons.

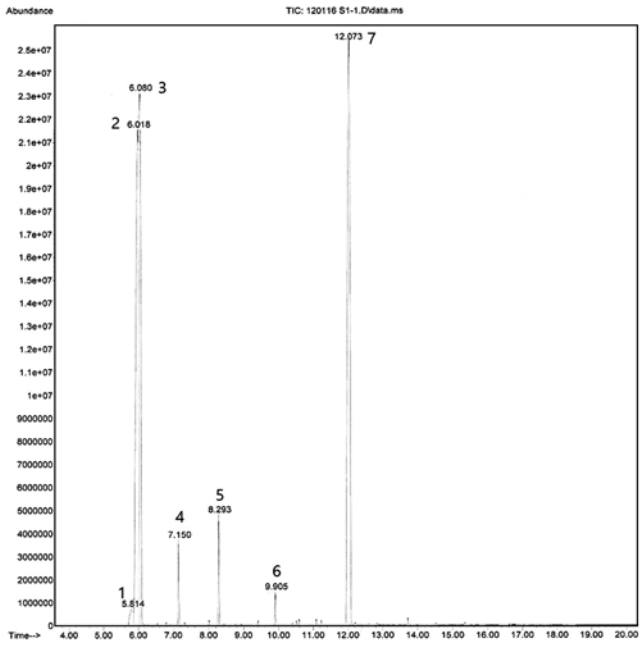
Results and Discussion

Active components of horseradish root ITCs antibacterial activities ITCs extracted from horseradish root were analyzed using GC-MS for identification of the components involved in antibacterial activities (Fig. 1). ITCs comprised 2 major ITCs derivatives of 59.995% AITC and 35.819% PITC, and a minor derivative of 1.532% 3-Butyl isothiocyanate (BITC). Concentrations of AITC and PITC in ITCs were calculated using a standard curve (data not shown). The extract contained 205,367 μg/mL of AITC and 71,559 μg/mL of PITC. The active components in horseradish root ITCs were different from active components in ITCs extracted from wasabi (*Wasabia japonica*), which contained 12 ITCs, but no PITC in the root, stem, or leaf (33).

Isolation and identification of dominant bacteria Fourteen different colony types were isolated from 3 *jeotgal* varieties (data not shown). Six colony types were isolated from squid *jeotgal* that grew from a 10,000× dilution (named S1-S5) and from a 100,000× dilution (named S6). Four colony types were isolated from *myeong-ran jeotgal* that grew from a 100,000× dilution (named M1-M4) and 4 colony types were isolated from *chang-ran jeotgal* that grew from a 100× dilution (named C1-C4). Six isolated strains that exhibited high population numbers on BHI agar were selected and identified to genus and species levels based on biochemical testing (Table 1). Six isolates (S4, S5, S6, M4, C2, and C3) from 3 kinds of *jeotgal* were identified as *Bacillus amyloliquefaciens* (S4 and C2), *Staphylococcus xylosus* (5), *Streptococcus* sp. (S6), *Enterobacter amnigenus* 2 (M4), and *Bacillus pumilus* (C3).

Antibacterial activities of ITCs based on the disc diffusion and disc volatilization methods Antibacterial activities of ITCs in the liquid phase (disc diffusion method) and the vapor phase (disc volatilization method) against 5 dominant bacteria are shown in Table 2. ITCs in the liquid phase showed the strongest antibacterial activities against *B. pumilus* with an inhibition zone ratio of 11.07±0.10, followed *B. amyloliquefaciens* (10.93±0.10) by disc diffusion method. ITCs in the liquid phase showed stronger antibacterial activities against Gram-positive bacteria, except for *Streptococcus* sp., than against Gram-negative bacteria, although *E. amnigenus* 2 was the only Gram-negative bacterium tested herein.

ITCs in the vapor phase showed a tendency similar to the antibacterial activities of ITCs in the liquid phase. The strongest antibacterial activities were observed against *B. pumilus* and *B. amyloliquefaciens* (14.50±0.00) by disc volatilization method. ITCs in the vapor phase also showed



Peak No.	RT (min)	Compound	Peak area (%)
1,2,3	5.814 /6.018 / 6.080	Allyl ITC (AITC)	59.995
4	7.150	3-Butenyl ITC (BITC)	1.532
5	8.293	Triethyl-2-propenyl	2.102
6	9.905	Benzene propanenitrile	0.588
7	12.073	Phenylethyl ITC (PITC)	35.819

Fig. 1. Analysis of ITCs extracted from horseradish root using GC-MS.

Table 1. Identification of 5 dominant bacteria isolated from 3 *jeotgal* varieties

Type of *Jeotgal*	Isolate	Genus/Species		Probability of identification (%)	Population (%)
		Biochemical test	Automated microbiology system		
Squid	S4	*Bacillus*	*Bacillus amyloliquefaciens*	98	5.0
	S5	*Staphylococcus*	*Staphylococcus xylosus*	98	5.3
	S6	*Streptococcus*	*Streptococcus* sp.	-[1)]	84.4
Myeong-ran	M4	*Enterobacter*	*Enterobacter amnigenus* 2	99	60.9
Chang-ran	C2	*Bacillus*	*Bacillus amyloliquefaciens*	98	50.0
	C3	*Bacillus*	*Bacillus pumilus*	99	25.7

[1)] unidentified by the automated microbiology system.

Table 2. Antibacterial activities of ITCs extracted from horseradish root against 5 dominant bacteria from *jeotgal* based on the disc diffusion and disc volatilization methods[1)]

Tested bacteria	Ratio of inhibition zone [(diameter of clear zone (mm) / diameter of paper disc (6 mm)]	
	Disc diffusion method	Disc volatilization method
Bacillus amyloliquefaciens (G +)	10.93±0.10^{aD}	14.50±0.00^{bD}
Bacillus pumilus (G +)	11.07±0.10^{aD}	14.50±0.00^{bD}
Staphylococcus xylosus (G +)	2.03±0.28^{aC}	5.32±0.28^{bC}
Streptococcus sp.(G +) [z]	1.21±0.35^{aA}	1.45±0.01^{bA}
Enterobacter amnigenus 2 (G -)	1.73±0.05^{aB}	4.02±0.32^{bB}

[1)] Mean (triplicate)±SD
[A-D]Means within the same column with different superscript upper case letters are different (p<0.05).
[a-b]Means within the same row with different superscript upper case letters are different (p<0.05).

Table 3. MIC and MBC values of ITCs extracted from horseradish root against 5 dominant bacteria from *jeotgal* based on the broth dilution and disc volatilization methods[1)]

Tested bacteria	Broth dilution (mg/mL)		Disc volatilization (mg/mL)	
	MIC	MBC	MIC	MBC
Bacillus amyloliquefaciens	0.156±0.000^{bA}	0.208±0.091^{aA}	0.039±0.000^{aA}	0.078±0.000^{aA}
Bacillus pumilus	0.156±0.000^{bA}	0.208±0.091^{aA}	0.039±0.000^{aA}	0.078±0.000^{aA}
Staphylococcus xylosus	0.261±0.091^{bA}	0.313±0.000^{bA}	0.039±0.000^{aA}	0.078±0.000^{aA}
Streptococcus sp.[2)]	1.667±0.722^{aB}	2.083±0.722^{aB}	1.563±0.625^{aB}	1.875±0.722^{aB}
Enterobacter amnigenus 2	0.313±0.000^{bA}	0.521±0.180^{bA}	0.052±0.023^{aA}	0.078±0.000^{aA}

[1)]Mean (triplicate)±SD.
[2)]MIC and MBC values of *Streptococcus* sp. are shown as a mean of 4 replications±SD.
[A-B]Means within the same column with different superscript upper case letters are different (p<0.05).
[a-b]Means of MIC and MBC within the same raw with different superscript upper case letters are different (p<0.05).

stronger antibacterial activities against Gram-positive bacteria, except for *Streptococcus* sp., than against the Gram-negative bacterium *E. amnigenus* 2. *B. amyloliquefaciens* and *B. pumilus* were completely inhibited (diameter of the clear zone=diameter of the Petri dish) by ITCs in the vapor phase. ITCs in the vapor phase showed a significantly (p<0.05) stronger antibacterial activity than in the liquid phase against all tested bacteria.

ITCs extracted from horseradish root exhibited multi-targeted mechanisms of action in metabolic pathways, membrane integrity, cellular structures, and a significantly greater release of microorganism cell compounds (34-36). Ahn *et al*. (37) reported that ITCs showed stronger antimicrobial activities against Gram-negative bacteria than against Gram-positive bacteria. In this study, however, ITCs extracted from horseradish root in both the liquid phase and the vapor phase showed stronger antimicrobial activities against Gram-positive bacteria than against Gram-negative bacteria, perhaps due to the differences in cell membrane constituents and structures. Gram-positive bacteria have a single cell membrane layer that is an ineffective permeability barrier. Therefore, ITCs can easily penetrate the cell membrane and interact with intracellular sites, causing cell death. Gram-negative bacteria have outer and inner cell membranes that contain lipopolysaccharides, which makes ITCs penetration difficult (24,25,38).

Ratios of ITCs inhibition zones in the vapor phase were significantly (p<0.05) higher than for ITCs in the liquid phase against all tested strains. Cavanagh and Wilkinson (39) reported that essential oils of *Lavandula angustifolia* and *Mentha piperita* in the vapor phase have a lethal effect against several pathogenic bacteria, even at small doses (MIC values of approximately 0.050 and 0.025 μL/mL of head space). Tyagi and Malik (25) reported that the antimicrobial activity of *Mentha piperita* oil in the vapor phase was stronger than in the liquid phase. ITCs in the vapor phase have a greater application potential than corresponding liquid phases. Integration of AITC has been attempted using an encapsulation technique for preservation of fresh-cut onions (40). The mechanism of ITCs in the vapor phase is mainly associated with cell membrane damage. The antibacterial activity of ITCs in the vapor phase is primarily due to more hydrophobic (water-insoluble) and volatile properties (32,41,42). Hydrophobic ITCs in the vapor phase can better penetrate and accumulate in lipid-rich areas of cell membrane structures and cause structural and functional damage.

MIC and MBC values of ITCs MIC and MBC values of ITCs against 5 dominant bacteria baed on the broth dilution (liquid phase) and disc volatilization methods (vapor phase) are shown in Table 3. MIC values of ITCs in

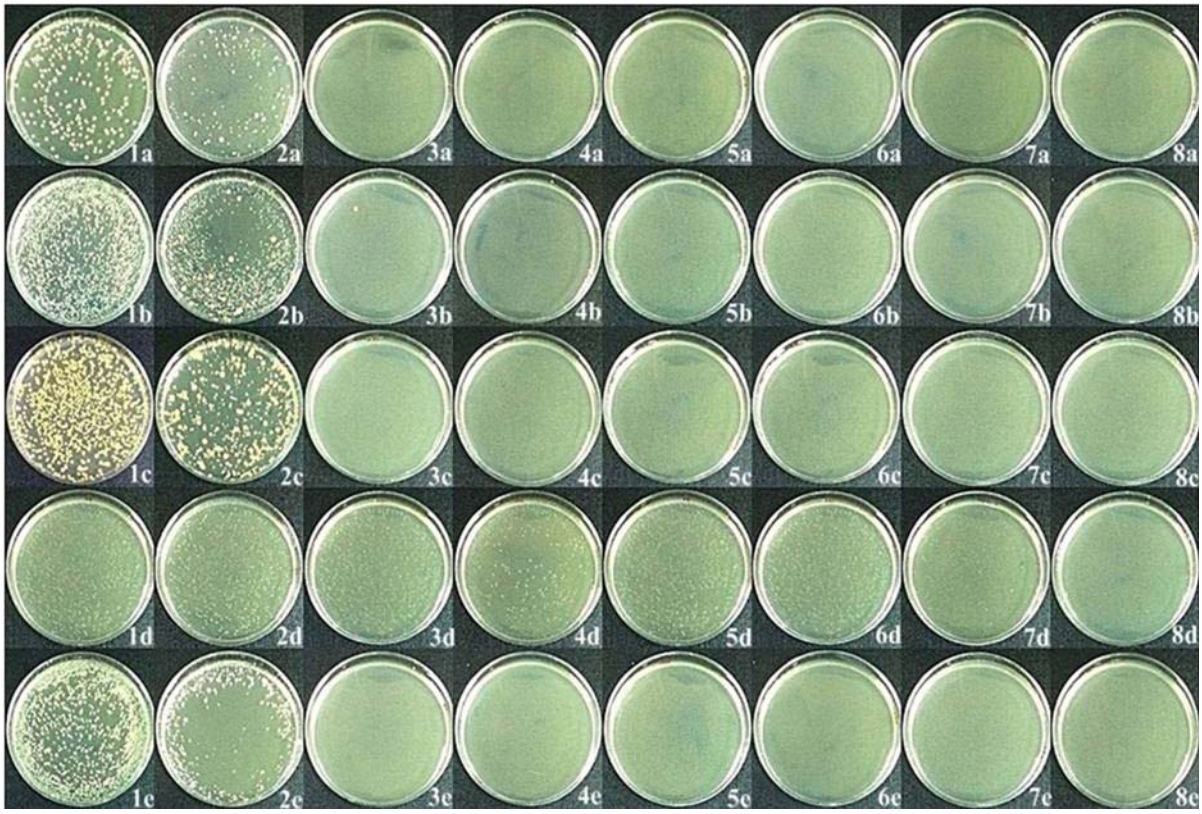


Fig. 2. Bactericidal activities of isothiocyanates (ITCs) extracted from horseradish (*Armoracia rusticana*) root in the vapor phase against *Bacillus amyloliquefaciens* (a 1-8), *Bacillus pumilus* (b 1-8), *Staphylococcus xylosus* (c 1-8), *Streptococcus* sp. (d 1-8), and *Enterbacter amnigenus* 2 (e 1-8) at 25°C. ITCs concentration: 1), 0.078 mg/mL; 2), 0.156 mg/mL; 3), 0.313 mg/mL; 4), 0.625 mg/mL; 5), 1.250 mg/mL; 6), 2.500 mg/mL; 7), 5.000 mg/mL; 8), 10.000 mg/mL.

the liquid phase against 5 dominant bacteria based on the broth dilution method ranged from 0.156±0.000 to 1.667± 0.722 mg/mL. MIC values of ITCs in the liquid phase against the Gram-positive bacteria *B. amyloliquefaciens* and *B. pumilus* and *S. xylosus* were not significantly (p>0.05) different from the Gram-negative *E. amnigenus* 2 value. The highest MIC value of 1.667±0.722 mg/mL for ITCs in the liquid phase was shown by *Streptococcus* sp. MBC values of ITCs in the liquid phase ranged from 0.208±0.091 to 2.083±0.722 mg/mL, and showed a pattern similar to MIC values. ITCs in the liquid phase showed the strongest antibacterial activities against *B. amyloliquefaciens* and *B. pumilus* with MBC values of 0.208±0.091 mg/mL.

MIC values of ITCs in the vapor phase (disc volatilization method) ranged from 0.039±0.000 to 1.563±0.625 mg/mL in the head space of agar plates. MIC values of ITCs in the vapor phase against the Gram-positive bacteria *B. amyloliquefaciens*, *B. pumilus*, and *S. xylosus* were not significantly (p>0.05) different from MIC values against the Gram-negative bacterium *E. amnigenus* 2. The highest MIC value of 1.563±0.625 mg/mL in the head space of ITCs in the vapor phase was shown by *Streptococcus sp.* MBC values of ITCs in the vapor phase ranged from 0.078±0.000 to 1.875±0.722 mg/mL in the head space (Fig. 2) and showed a pattern similar to MIC values. All tested bacterial strains with MBC values of 0.078±0.000 mg/mL in the head space were sensitive to ITCs in the vapor phase, except *Streptococcus* sp., with an MBC value of 1.875±0.722 mg/mL in the head space.

MIC and MBC values of ITCs against Gram-positive bacteria, except *Streptococcus* sp., were not significantly (p>0.05) different from the Gram-negative bacterium (*E. amnigenus* 2) based on both methods, although Gram-positive bacteria were more sensitive to ITCs than the Gram-negative bacterium based on ratio values of inhibition zones. Kloucek *et al*. (26) studied antimicrobial activities of vapors from *A. rusticana* against several food-borne disease bacteria, including *Salmonella enteritidis* (G–), *Staphylococcsu aureus* (G+), and *Pseudomonas aeruginosa* (G–) and reported the same MIC value of 0.031 mg/mL in the head space for both Gram-positive and Gram-negative bacteria, approximately the same as reported herein (0.039 mg/mL in the head space). In addition, Nedorostova *et al*. (24) reported similar results in which ITCs in the vapor phase at the same concentration of 0.0083 μg/mL in the head space inhibited both Gram-positive and Gram-negative food-borne disease bacteria. In contrast to this study, Ogawa *et al.* (43) reported that Gram-negative bacteria

were more sensitive to ITCs than Gram-positive bacteria.

ITCs extracted from horseradish roots showed antibacterial activities against both Gram-positive and Gram-negative bacteria isolated from *jeotgal*. However, *Sterptococcus* sp. is a lactic acid bacterium that was only weakly inhibited by ITCs. MIC and MBC values of ITCs against *Streptococcus* sp. from both methods were higher than for other strains (Table 3). Kyung and Fleming (44) reported that a higher concentration of AITC was required to inhibit *Lactobacillus plantarum* and *Lactobacillus brevis* than to inhibit *L. monocytogenes, S. aureus* and *E. coli* in an aqueous medium, and that *Lactobacillus agilis* R16 was initially inhibited by AITC, but resumed growth after 72 h at 30ºC (45). The combined effects of ITCs and competition from lactic acid bacteria can prevent growth of spoilage and pathogenic bacteria in fermented foods (46). Further testing of the antibacterial activities of the ITCs extracted from horseradish root against *Streptococcus* sp. is needed to better understand the actions of lactic acid bacteria.

In this study, antibacterial activities of ITCs in the vapor phase extracted from horseradish root against the 5 dominant bacteria *B. amyloliquefaciens*, *B. pumilus, S. xylosus, Streptococcus* sp., and *E. amnigenus* 2 isolated from squid, *myeong-ran* and *chang-ran jeotgal* 3 *jeotgal* were identified. ITCs can be used as a natural preservative, even at low concentrations, to extend the shelf-life of *jeotgal*.

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Disclosure The authors declare no conflict of interest.

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