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Antagonistic and antioxidant effect of probiotic *Weissella cibaria* JW15

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Abstract The objective of this study was to evaluate the functional properties of *Weissella cibaria* JW15 (JW15) by investigating its antagonistic and antioxidant activities. *Lactobacillus rhamnosus* GG (LGG) was used for comparison as a reference strain. JW15 inhibited the growth of pathogenic bacteria (*Listeria monocytogenes*, *Salmonella* Typhimurium, *S.* Enteritidis, and *Escherichia coli*). Compared to LGG, JW15 showed rapid organic acid production, with the amounts of lactic and acetic acids being lower and higher, respectively. In addition, JW15 significantly inhibited intestinal epithelial adherence in the tested pathogens. JW15 exhibited antioxidant effects by scavenging radicals including DPPH, ABTS, and hydroxyl radicals, and inhibiting lipid peroxidation. JW15 exhibited significant antagonistic and antioxidant activities compared to LGG in the tested assay ($p < 0.05$). The results suggested that JW15 might possess a potential for amelioration of disorders induced by pathogenic bacteria or oxidative stress.

Keywords *Weissella cibaria* JW15 · Probiotic · Antagonistic · Antioxidant

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Introduction

Lactic acid bacteria (LAB), the traditional probiotic strain, provide the host with health benefits through intestinal colonization. As LAB are used for the development of prophylactic and therapeutic treatments for complex health disorders, and therefore, their functional capacity and bacterial composition is an important consideration (Lebeer et al., 2012; Medzhitov, 2007).

Infections by various pathogens are a crucial public health concern; however, the abuse of antibiotics causes the emergence of antibiotic-resistant pathogen (Shi et al., 2013). Therefore, LAB-mediated antimicrobial properties are an effective option for substitution of antibiotics. LAB typically develop antagonistic activity against various human pathogens through the production of antimicrobial compounds, such as organic acids and bacteriocin (Bernet et al., 1994; De Vuyst and Leroy, 2007).

Reactive oxygen species (ROS), the oxidative free radicals, are unavoidably generated during cellular metabolism. Although ROS mediate various biological functions, an imbalance between the generation and elimination of ROS induces oxidative stress and damage of biomolecules (Li et al., 2012). Since endogenous antioxidant capacity is limited, exogenous supplementation is required to regulate the oxidative stress (Fang et al., 2002).

The genus *Weissella*, a relatively recent member of the LAB family, comprises 23 species, including *W. kimchii*, *W. koreensis*, and *W. cibaria* (Fusco et al., 2015). In particular, *W. cibaria* was dominantly derived from kimchi in Korea. Previous studies demonstrated that *W. cibaria* JW15 derived from kimchi exhibited probiotic properties and immuno-modulatory effects (Ahn et al., 2013; Lee et al., 2018).

The objective of the present study was to investigate the functional effects of the potential probiotic strain, *W. cibaira* JW15. Antagonistic activity against pathogens and antioxidant activity of intact bacterial cells were evaluated.

Materials and methods

Bacterial strains and maintenance

Weissella cibaria KACC 91811P (JW15) was obtained from the Korean Agricultural Culture Collection (KACC; Jeollabuk-do, Korea). *Lactobacillus rhamnosus* KCTC 12202BP (LGG) was obtained from the Korean Collection for Type Cultures (KCTC; Daejeon, Korea) and used as a reference strain. The indicator pathogenic strains were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). LAB and pathogenic strains were incubated and maintained in De Man, Rogosa and Sharpe (MRS) broth and tryptic soy broth (TSB) (Difco Laboratories, Detroit, MI, USA), respectively, at 37 °C.

Antimicrobial activity

The LAB-mediated antimicrobial activity was assessed with deferred antagonism method as described by Makras and De Vuyst (2006) with minor modifications. An overnight culture of each LAB strains was spotted on MRS agar and incubated for 24 h at 37 °C. Next, the MRS agar was overlaid with 10 mL of soft TSA containing the indicator pathogen (5×10^7 CFU/mL). The plate was incubated for another 24 h at 37 °C. The radius of the developed clear zone was measured.

Organic acid production

Organic acid production of LAB strains was determined by HPLC. The supernatant of bacterial culture was filtered (0.45 μm) and injected (20 μL). Analysis was performed by using the Agilent 1100 apparatus (Agilent Technologies, Santa Clara, CA, USA) equipped with a Bio-Rad Aminex HPX-87H column (300 × 7.8 mm, 5 μm). The column was maintained at 50 °C and 5 mM H_2SO_4 was employed as the eluent at a flow rate of 0.6 mL/min. The chromatogram was detected with a UV-detector at 210 nm.

Anti-adhesion of pathogenic strains to intestinal cells

LAB-mediated inhibition of pathogenic bacteria adherence to intestinal epithelial cells were determined as described by Lee et al. (2015) with minor modification. Briefly, Caco-2 cell line was seeded in 24-well plate (1×10^5 -cells/well) and incubated until monolayers were formed. Each of the LAB and pathogenic strains were diluted (1×10^8 CFU/mL). An aliquot (100 μL) of the pathogenic strain with or without the LAB was added to wells and incubated for 2 h. The cells were washed thrice with PBS and lysed with 0.1% Triton X-100. Cell lysates were spread on selective agar (Oxford, EMB, and XLD) and incubated for 24 h. The anti-adhesion rate was assessed as follows:

$$\text{Anti-adhesion rate}\,(\%) = (1 - N_1/N_0) \times 100$$

where N_0 was the numbers of pathogenic bacteria without LAB strains, and N_1 was the number of pathogenic bacteria with LAB strains.

Antioxidant activity

Radical scavenging activity of intact LAB cells were determined using DPPH, ABTS, and hydroxyl radicals. Each of LAB strains was suspended in PBS (1.0×10^9 CFU/mL). DPPH solution was prepared by dissolving DPPH in ethanol (100 μM). An aliquot (200 μL) of the bacterial suspensions or PBS were mixed with 1 mL of a DPPH solution and incubated at room temperature for 20 min in dark. The absorbance of each samples was measured at 517 nm.

ABTS stock solution was prepared by mixing equal volumes of 7.4 mM ABTS and 2.6 mM potassium persulfate at room temperature for 16 h in the dark. The ABTS stock solution was then diluted with PBS to obtain an absorbance of 0.70 ± 0.05 at 734 nm. Next, an aliquot (100 μL) of bacterial suspensions or PBS was mixed with 1 mL of an ABTS solution and incubated at room temperature for 20 min in dark. The absorbance of samples was measured at 734 nm.

The hydroxyl radical scavenging activity was determined with deoxyribose method described as Siddhuraju and Becker (2007). An aliquot (1 mL) of 20 mM phosphate buffer (pH 7.4) containing deoxyribose (2.8 mM), EDTA (0.1 mM), and ferric chloride (0.1 mM) was mixed with an equal volume of bacterial suspension. Next, 0.1 mL of ascorbic acid (1 mM) and 0.5 mL of hydrogen peroxide (20 mM) were added. The mixture was incubated at 37 °C for 1 h. Following treatment of 0.3 mL 2-thiobarbituric acid (100 mM) and 1 mL trichloroacetic acid (60 mM), the mixture was incubated at 95 °C for 30 min. The absorbance was measured at 532 nm. The radical scavenging activity was calculated as follows:

$$\text{Radical scavenging activity}\,(\%) = [1 - (A_S/A_C)] \times 100$$

where A_S was absorbance of the sample and A_C was absorbance of the control.

Inhibition effect of LAB on lipid peroxidation was determined using the β-carotene-linoleate system as described by Son et al. (2017) with modifications.

Chloroform containing β-carotene (2 mg), linoleic acid (44 μL), and Tween 80 (200 μL) was subjected to evaporation at 45 °C in a vacuum. The β-carotene emulsion was prepared by addition of 100 mL of distilled water. The emulsion (4 mL) was mixed with bacterial suspension (1 mL) and incubated at 50 °C for 4 h. The absorbance was measured at 470 nm. The antioxidant activity was calculated as follows:

$$\text{Antioxidant activity}\,(\%) = [1-(S_{4\,h}-S_{0\,h})/(C_{4\,h}-C_{0\,h})] \times 100$$

where $S_{4\ h}$ and $S_{0\ h}$ were the absorbance of sample at 4 h and 0 h, respectively, and $C_{4\ h}$ and $C_{0\ h}$ were the absorbance of control at 4 h and 0 h, respectively.

Statistical analysis

The results were presented as mean ± SD from triplicate of independent experiments. The unpaired one-tailed Student's t test was performed to analyze the statistical difference between two groups using IBM SPSS version 24.0 for Windows version (SPSS Inc., Chicago, IL, USA). $p < 0.05$ considered as statistically significant.

Results and discussion

Antimicrobial activity and organic acid production

The LAB-mediated antagonistic activity against a wide range of human pathogens is one of the most well documented beneficial properties. In present study, LAB-derived antimicrobial activities against major foodborne pathogens were determined by the deferred antagonism method (Table 1). JW15 significantly inhibited the growth of the tested pathogens ranging from 20.5 to 26.2 mm. Moreover, JW15 inhibited the growth of various pathogenic bacteria including *Staphylococcus aureus*, *Streptococcus mutans*, *Campylobacter jejuni*, and *Vibrio parahaemolyticus* (data not shown). The inhibitory activity was more effective on gram-positive bacteria than gram-negative bacteria. In addition, JW15 exhibited statistical significance in antimicrobial activity compared to LGG ($p < 0.05$).

To gain more insight of the antimicrobial activity, the organic acid production profile of LAB strains was determined by HPLC (Table 2). Compared to LGG, JW15 exhibited rapid organic acid production; however, the concentrations of lactic and acetic acids were lower and higher, respectively, after 24 h of incubation. Correlated with these results, probiotic strains, including *Lactobacillus* spp. and *Bifidobacterium* spp., exerted antimicrobial activities against pathogens via the production of lactic and acetic acids (Tejero-Sariñena et al., 2012). Similarly, LGG exerts antimicrobial activity against *Salmonella* Typhimurium by accumulating the lactic acid (De Keersmaecker et al., 2006). The organic acids have been utilized as natural preservatives due to their broad antimicrobial and antifungal activities by inducing the malfunction of cellular metabolism. Additionally, acetic acid has been known to be the most effective antimicrobial organic acid but also exerts the synergistic effect with lactic acid (De Vuyst and Leroy, 2007; Peláze et al., 2012).

Anti-adhesion to intestinal epithelial cells

LAB-mediated anti-adhesion activity against pathogenic bacteria was assessed in an intestinal epithelial cell line (Table 1). JW15 inhibited the adhesion of the tested pathogenic bacteria ranging from 55.43 to 69.44%. JW15 showed the highest inhibitory effect on *L. monocytogenes*. Furthermore, JW15 exhibited statistical significance for its anti-adhesion effect compared to LGG ($p < 0.05$). Generally, LAB exhibited antagonistic activity by inhibiting the adherence and colonization of pathogens in gastrointestinal tract (GIT). Previous studies have reported that

Table 1 Determination of antimicrobial and anti-adhesion effects of lactic acid bacteria (LAB) strains against pathogenic strains

Assays	LAB strains	Pathogenic strains			
		Listeria monocytogenes ATCC15313	*Salmonella* Typhimurium ATCC 23564	*Salmonella* Enteritidis ATCC 13076	*Escherichia coli* ATCC 10536
Antimicrobial activity (mm)	LGG[A]	24.3 ± 0.85[a]	18.3 ± 1.03[a]	20.8 ± 1.31[a]	21.2 ± 0.85[a]
	JW15[B]	26.2 ± 0.62[b]	20.5 ± 1.08[b]	21.7 ± 0.62[a]	24.2 ± 0.47[b]
Anti-adhesion rate (%)	LGG[A]	50.71 ± 4.94[a]	49.55 ± 7.99[a]	44.01 ± 8.33[a]	51.39 ± 5.38[a]
	JW15[B]	69.44 ± 8.69[b]	57.74 ± 8.09[b]	55.43 ± 7.22[b]	63.13 ± 6.32[b]

All values are presented as mean ± SD from triplicate of independent experiments

[A]*Lactobacillus rhamnosus* GG; [B]*Weissella cibaria* JW15

[a,b]Different superscripts in the same column represent statistical significance based on unpaired one-tailed Student's t test ($p < 0.05$)

Table 2 Organic acid production profile of lactic acid bacteria (LAB) strains during incubation in MRS broth

Organic acid	LAB strains	Concentration (g/L)				
		0 h	2 h	6 h	12 h	24 h
Lactic acid	LGG[A]	ND	0.10 ± 0.01[a]	0.36 ± 0.01[a]	6.16 ± 0.47[a]	17.23 ± 0.98[b]
	JW15[B]	ND	0.16 ± 0.01[b]	1.28 ± 0.08[b]	8.06 ± 0.26[b]	12.60 ± 0.31[a]
Acetic acid	LGG	ND	ND	0.07 ± 0.01[a]	0.27 ± 0.03[a]	0.57 ± 0.04[a]
	JW15	ND	ND	0.36 ± 0.03[b]	1.08 ± 0.11[b]	1.30 ± 0.05[b]

All values are presented as mean ± SD from triplicate of independent experiments

ND not detected

[A]*Lactobacillus rhamnosus* GG; [B]*Weissella cibaria* JW15

[a,b]Different superscripts in the same column represent statistical significance based on unpaired one-tailed Student's t test ($p < 0.05$)

Table 3 Antioxidant activity of lactic acid bacteria (LAB) strains in various models

Antioxidant assays	LAB strains	
	LGG[A]	JW15[B]
DPPH radical scavenging activity (%)	18.88 ± 1.57[a]	23.53 ± 0.77[b]
ABTS radical scavenging activity (%)	49.03 ± 2.46[a]	60.85 ± 3.75[b]
Hydroxyl radical scavenging activity (%)	39.30 ± 6.57[a]	51.66 ± 7.18[b]
Inhibition rate of lipid peroxidation (%)	17.50 ± 4.22[a]	33.31 ± 4.53[b]

All values are presented as mean ± SD from triplicate of independent experiments

[A]*Lactobacillus rhamnosus* GG; [B]*Weissella cibaria* JW15

[a,b]Different superscripts in the same row represent statistical significance based on unpaired one-tailed Student's t test ($p < 0.05$)

Lactococcus lactis KC24 inhibited intestinal adherence of *L. monocytogenes* and *S. aureus*. Similarly, *L. reuteri* suppressed the adherence of *Helicobacter pylori* through the inhibition of binding to the glycolipid receptors (Lee et al., 2015; Mukai et al., 2002).

Antioxidant activity

LAB strains exhibit resistance against ROS-induced oxidative stress by expressing the antioxidant enzymes, such as super oxide dismutase, glutathione reductase, and NADH peroxidase (Tang et al., 2017). To determining the antioxidant effect, LAB-mediated radical scavenging and inhibition of lipid peroxidation activities were evaluated (Table 3). Intact JW15 cells showed radical scavenging effect ranging from 23.53 to 60.85% and inhibited lipid peroxidation of 33.31% in the tested assays. Additionally, JW15 exhibited a statistical difference on the antioxidant effect compared to LGG. Excessive ROS generation may cause the oxidative stress-induced cellular malfunction and result in various disorders. Several studies have reported probiotics-mediated antioxidant effect, such as free radical scavenging activities and up-regulated antioxidant enzyme expressions against H_2O_2-induced stress. Therefore, LAB-mediated antioxidant activity in GIT may contribute to the maintenance of health via oxidative stress regulation (Son et al., 2017; Tang et al., 2017).

In conclusion, JW15 was confirmed to be a potential functional probiotic strain with antagonistic activity against pathogens and antioxidant effect. JW15 exhibited organic acid production-mediated antimicrobial activity and inhibition of pathogen adherence. JW15 showed antioxidant effects via scavenging of free radicals and inhibition of lipid peroxidation. While further investigations are required to illustrate the mechanisms of functional properties, JW15 could be considered as a potential prophylactic probiotic strain with applications in the food industry.

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Compliance with ethical standards

Conflict of interest The authors have declared no conflict of interest.

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