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Anti-bacterial and anti-inflammatory activities of lactic acid bacteria-bioconverted indica rice (*Oryza sativa* L.) extract

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Abstract

Background: In this study, indica rice (*Oryza sativa* L.) was bioconverted using *Lactobacillus plantarum* ATCC14917 and *Lactobacillus rhamnosus* GG KCTC5033.

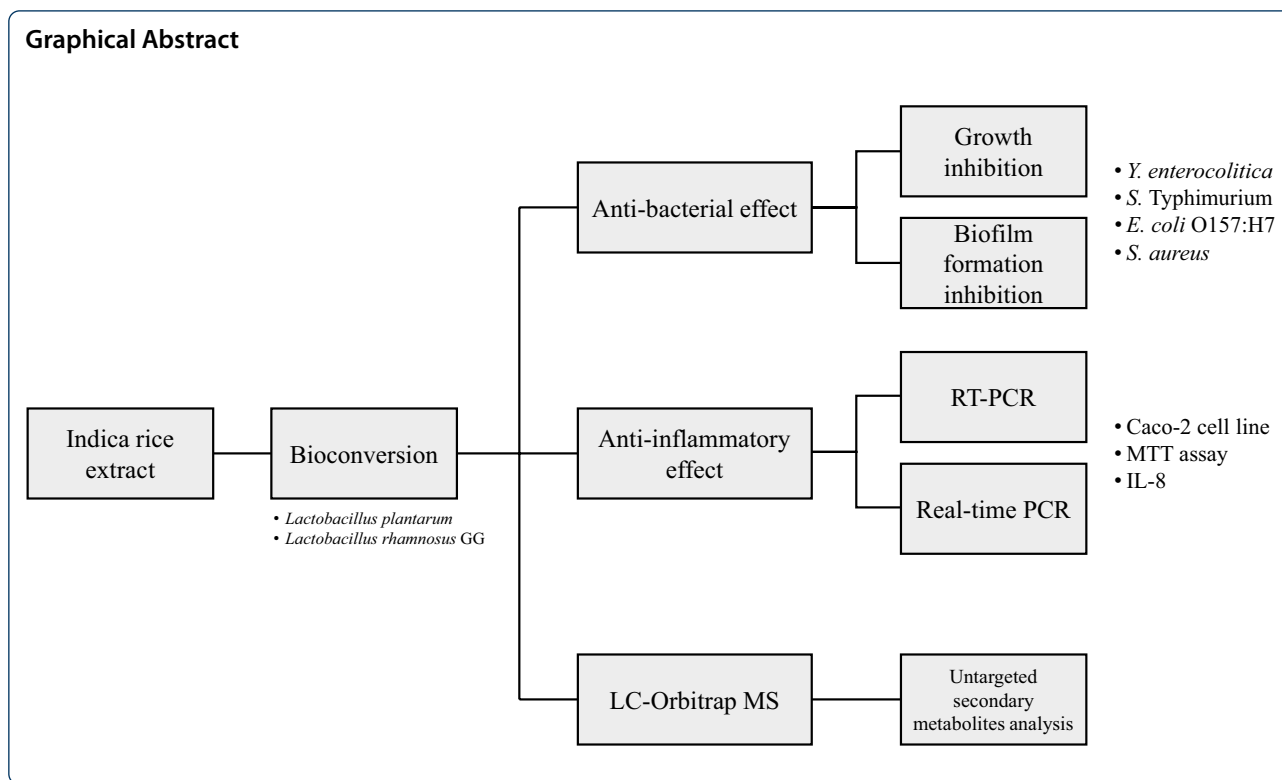
Results: Compared to the control treatment (RE), rice extract fermented with *Lactobacillus plantarum* ATCC14917 (LP-RE) and *Lactobacillus rhamnosus* GG KCTC5033 (LR-RE) inhibited the growth of pathogenic bacteria significantly except for *E. coli* O157:H7 ($p < 0.05$). The LR-RE treatment had the strongest inhibitory effect on *Staphylococcus aureus* biofilm formation ($10.81 \pm 1.98\%$). Additionally, real-time PCR analyses confirmed that LP-RE inhibited *S. aureus*-induced IL-8 expression in Caco-2 cells by 60%. HPLC-orbitrap analysis indicated that this treatment increased the levels of key metabolites with therapeutic properties such as acetophenone, 9-oxo-10(E), 12(E)-octadecadienoic acid, nicotinic acid, and indol-3-lactic acid.

Conclusions: This study demonstrated that the secondary metabolites were linked to antibacterial and anti-inflammatory effects, suggesting that bioconversion is a promising means to produce dietary supplements with therapeutic properties.

Keywords: Indica rice, Bioconversion, Lactic acid bacteria, Anti-bacterial, Anti-inflammatory, Secondary metabolite

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Introduction

Nutrient intake not only prevents or treats diseases, but also improves overall health [1]. However, contrary to consumer expectations, obtaining enough nutrients through dietary intake alone is often not possible in our busy modern society. Therefore, there is a rise in the consumer demand for novel nutrient-dense foods and supplements, which has led to the recent expansion of the health functional food industry [2]. Health functional food is defined as food manufactured and processed using ingredients that benefit the human body [3], and increasingly more people are consuming these products to prevent diseases rather than treating them with medicine.

Bioconversion is also often referred to as bioprocessing, biotransformation, biosynthesis, and biocatalysis, and these terms are generally used to describe the technology used for manufacturing products using microorganisms [4]. The bioconversion process differs from existing fermentation processes in that the fermentation process starts from relatively simple raw materials, whereas the bioconversion process produces products from materials by taking advantage of the substrate selectivity of microorganisms or enzymes. In this regard, the bioconversion process is known to be an energy-saving and high-tech sector that has advanced fermentation technology. Particularly, this process can be used in various ways to

improve the usefulness and effectiveness of medicines and cosmetics [5].

Biotechnology techniques that employ several lactic acid bacteria are promising alternatives for the establishment of cost-effective, renewable, and virtually inexhaustible sources of high-value aromatic and bioactive compounds. Bioactive compounds possess a variety of therapeutic properties such as antibacterial, anti-inflammatory, and antioxidant activities, as well as anti-wrinkle and anti-whitening properties. These properties can potentially improve various aspects of the food, pharmaceutical, chemical, and agrochemical industries. As mentioned earlier, the bioconversion method has a wide variety of applications [6].

Indica rice (*Oryza sativa* L.), also called Annammi in Korea, is a representative rice variety that accounts for 90% of the world's rice trade and is mainly grown in Southeast Asia. The name "indica" refers to the fact that this rice variety is widely consumed in India. Indica rice is an important cereal and is known to be highly adaptable to environmental stress [7]. Although indica rice contains several bioactive substances, there is a lack of knowledge regarding the health-promoting properties of bioconverted indica rice. A previous study reported the bioconversion of indica rice via inoculation with *Agrobacterium tumefaciens* [8]. Furthermore, although lactic acid bacteria-bioconverted mulberries (*Morus alba* L.) have

been reported to exert antimicrobial and anti-inflammatory activities [9], very few studies have focused on characterising lactic acid bacteria-bioconverted indica rice. In this study, the antimicrobial, anti-inflammatory, antioxidant, anti-wrinkle, and anti-whitening effects of indica rice bioconverted using *Lactobacillus plantarum* and *L. rhamnosus* GG were carried out.

Materials and methods

Bacterial culture conditions

Lactobacillus plantarum ATCC 14917, *Escherichia coli* O157:H7 ATCC 35150, *Yersinia enterocolitica* ATCC 23715, and *Staphylococcus aureus* ATCC 29213 were purchased from the American Type Culture Collection (Manassas, VA, USA). *Lactobacillus rhamnosus* GG KCTC 5033 and *Salmonella* Typhimurium KCTC 1925 were obtained from the Korean Collection for Type Culture (Jeongup, Korea). *L. plantarum* ATCC 14917 and *L. rhamnosus* GG KCTC 5033 were cultured in de Man Rogosa Sharpe (MRS) medium (Neogen, Lansing, MI, USA). *E. coli* O157:H7 ATCC 35150 and *S. Typhimurium* KCTC 1925 were cultured in Luria–Bertani (LB) medium (BD Biosciences, Franklin Lakes, NJ, USA). *Y. enterocolitica* ATCC 23715 and *S. aureus* ATCC 29213 were cultured in Tryptone Soy (TS) medium (BD Biosciences) and Brain Heart Infusion [10] (BD Biosciences) medium, respectively. All strains were incubated at 37 °C for 24 h.

Rice extract bioconversion process by *L. plantarum* and *L. rhamnosus* GG

Indica rice (*Oryza sativa* L.) was purchased at a local market (Seoul, Korea) and ground using a food grinder (SMKANB-4000, Poongnyun Co., Ltd., Korea). The rice was then immersed in distilled water at a ratio of 1:2.5 and boiled at 55 °C for 12 h in a water bath. Afterwards, the solid content was filtered through a sterilised cotton cloth and the pH of the supernatant was adjusted to 6.5–6.8. The rice extracts (RE) were then autoclaved prior to inoculation. Next, the extracts were inoculated with the bacterial strains (*L. plantarum* and *L. rhamnosus* GG; 1%, w/w) and incubated at 37 °C for 72 h. After incubation, the supernatants were collected by centrifugation at 10,170×g for 10 min and their pH was adjusted to 6.5–6.8. The *L. plantarum* (LP-RE) and *L. rhamnosus* GG (LR-RE) bioconverted RE was recovered by filtering the fermented samples through a syringe filter (0.25 µm, Advantec Dismic, ToyoRochi, Tokyo, Japan). Non-bioconverted RE was obtained by incubating the RE without *L. plantarum* and *L. rhamnosus* GG inoculation under the same conditions.

Anti-bacterial effects

Bactericidal activity of bioconverted RE

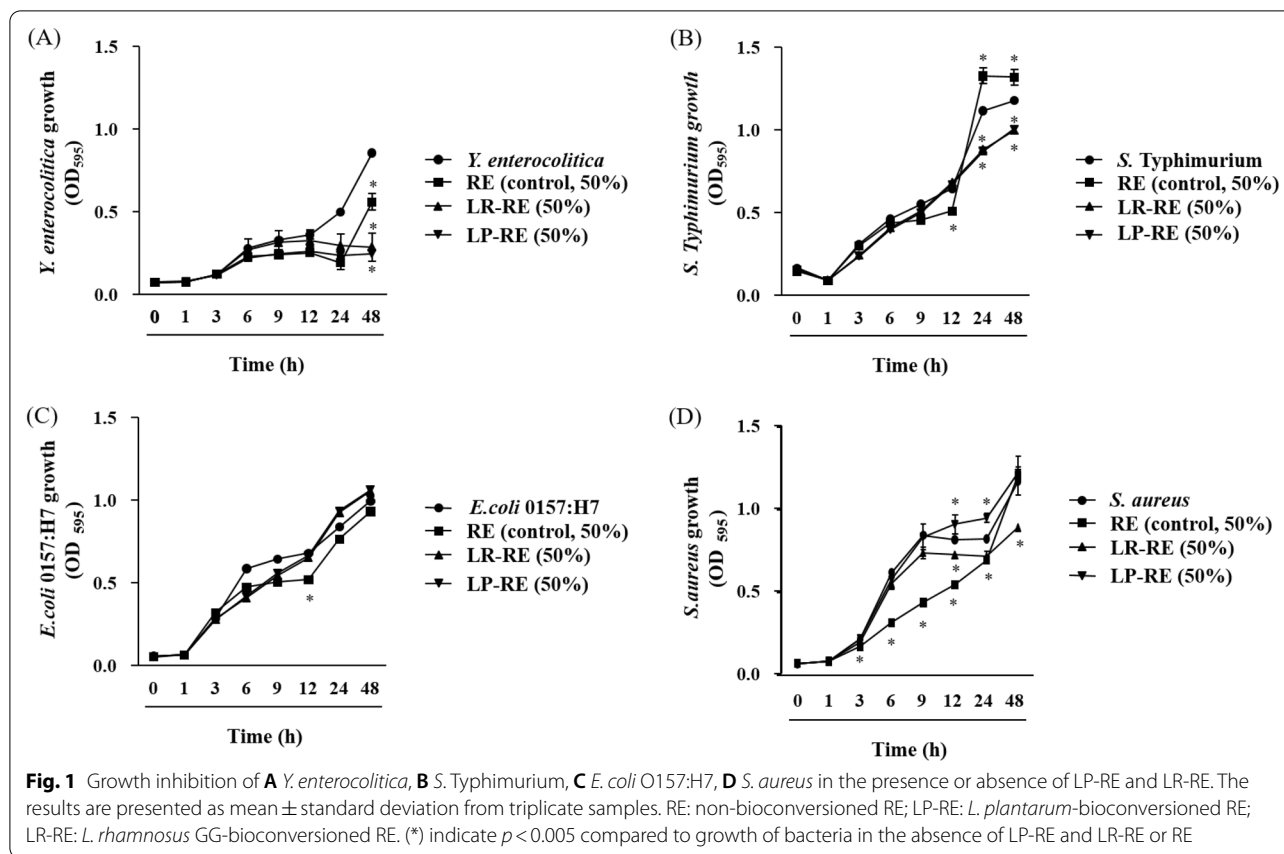
Yersinia enterocolitica, *S. Typhimurium*, *E. coli* O157:H7, or *S. aureus* were incubated with or without LP-RE or LR-RE at 50% in each appropriate broth media at 37 °C for 1, 3, 6, 9, 12, 24, and 48 h. These pathogenic bacteria were also incubated with RE under the same conditions. At the indicated incubation times, the growth of the bacteria was determined by measuring the optical density of the samples at 595 nm using a microtiter plate reader (Allsheng, Hangzhou, China).

Effect of bioconverted RE on pathogenic bacteria biofilm formation

Pathogenic bacteria (*Y. enterocolitica*, *S. Typhimurium*, *E. coli* O157:H7, and *S. aureus*) were cultured at 37 °C for 24 h and diluted with fresh broth to 1×10^7 CFU/mL. Next, 100 µL of the bacterial suspension was incubated in 96-well culture plates with or without LP-RE or LR-RE (0, 3.125, 6.25, 12.5, 25, and 50%, v/v) at 37 °C for 24 h. After incubation, the culture supernatants were removed and the plates were gently washed with phosphate-buffered saline (PBS). The biofilms formed by pathogenic bacteria were stained with crystal violet (0.1%) at room temperature for 30 min and the excess stain was removed by washing with PBS. The adherent stain was dissolved by adding 95% ethanol and 0.1% acetic acid, after which the optical density of the samples was measured at 595 nm.

Cell viability of Caco-2 cells in the presence of bioconverted RE

The cytotoxicity of the bioconverted RE was evaluated via the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Caco-2 cells, a human intestinal epithelial cell line, were purchased from the ATCC and cultured in complete Dulbecco's modified Eagle's medium (DMEM, HyClone, Logan, UT, USA) with 10% foetal bovine serum (Gibco, Burlington, ON, Canada), 100 U/mL penicillin, and 100 µg/mL streptomycin (HyClone, Logan, UT, USA) at 37 °C in a humidified 5% CO₂ and 95% air atmosphere. Caco-2 cells (5×10^5 cells/mL) were plated in 96-well culture plates and grown until fully confluent. The cells were then incubated in LP-RE, LR-RE, or RE (10%, v/v) at 37 °C for 24 h. After incubation, 10 µL of MTT (5 mg/mL) was added to 90 µL of cell suspensions and incubated at 37 °C for 4 h. The culture supernatants were removed and 100 µL of DMSO was added to each well. The plates were then shaken to dissolve the reduced intracellular formazan. The absorbance was measured at 570 nm.



Inhibitory effect of bioconverted RE on *S. aureus*-induced IL-8 in Caco-2 cells

Caco-2 cells (5×10^5 cells/mL) were plated in 6-well culture plates and grown until fully confluent as described above. The cells were co-incubated with *S. aureus* (1×10^7 CFU/mL) in LP-RE, LR-RE, or RE (0.1%, v/v) for 3 h. Total RNA was extracted using the TRIzol reagent (Invitrogen, Grand Island, NY, USA) and complementary DNA (cDNA) was synthesised using total RNA (2 μ g), reverse transcriptase (Promega, Madison, WI, USA), and random hexamers. The resulting cDNA was quantified via real-time quantitative reverse-transcription PCR (qRT-PCR) with SYBR Green Realtime PCR Master Mix (Toyobo, Osaka, Japan) using a Bio-Rad CFX thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions. The sequences of the specific IL-8 and β -actin primers were as follows: IL-8 (forward: 5'-GTG CAG TTT TGC CAA GGA GT-3', reverse: 5'-CTC TGC ACC CAG TTT TCC TT-3') and β -actin (forward: 5'-TGT TCG AGA CCT TCA ACA CG-3', reverse: 5'-ATC CCC AGA GTC CAT GAC AA-3'). The relative quantification of IL-8 mRNA expression was normalised to that of β -actin via the $2^{-\Delta\Delta C_t}$ method.

Profiling of secondary metabolites of bioconverted RE using LC-Orbitrap MS

Next, 50 μ L of test sample were extracted with 375 μ L of MeOH, 375 μ L of IPA, and 125 μ L of distilled water, after which the samples were sonicated for 10 min. After sonication, the samples were centrifuged at $16,363 \times g$ (Eppendorf centrifuge 5424R, Eppendorf AG, Hamburg, Germany) for 5 min at 4 $^{\circ}$ C. Evaporation was then conducted using 1000 μ L of supernatant. The test sample was reconstituted with 100 μ L of 80% MeOH prior to analysis. The metabolites were separated using a Vanquish system (Thermo Fisher Scientific, MA, USA) equipped with a Waters Acquity UPLC BEH C18 column (2.1 mm \times 150 mm, 1.7 μ m). The mobile phase consisted of water with 0.1% formic acid (solvent A, v/v) and acetonitrile with 0.1% formic acid (solvent B, v/v) at a 300 μ L/min flow rate. The gradient elution program was as follows: 0–2.0 min, 5% B; 2.0–25.0 min, 5–95% B; 25.0–27.5 min, 95% B; 27.5–27.6 min, 95–5% B; 27.6–30.0 min, 5% B. All samples were analysed using a Q-Exactive Focus instrument (ThermoFisher Scientific, MA, USA) equipped with an electrospray ionisation [11] interface (HESI-II) in positive mode, and the system was controlled using the Xcalibur 4.0 and Q-Exactive

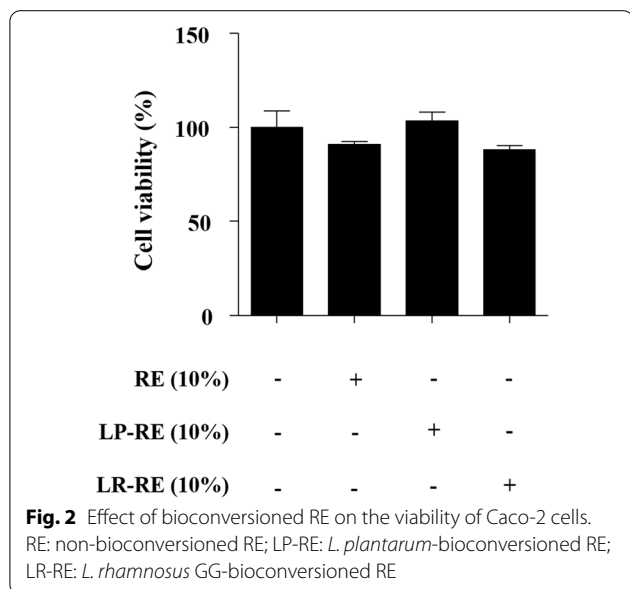
Table 1 Effect of lactic acid bacteria-bioconverted RE on the biofilm formation of bacteria (*S. Typhimurium*, *Y. enterocolitica*, *E. coli* O157:H7, *S. aureus*) at different concentrations (3.125, 6.25, 12.5, 25 and 50% v/v)

Bacteria	Sample	Biofilm formation (%) ^a					
		Control ^b	3.125%	6.25%	12.50%	25%	50%
<i>Y. enterocolitica</i>	RE ¹	100 ± 3.39 ^{aC}	90.35 ± 9.79 ^{efgC}	72.82 ± 3.21 ^{dB}	68.35 ± 4.02 ^{efB}	60.97 ± 1.05 ^{CB}	38.25 ± 5.29 ^{bCA}
	LP-RE	100 ± 4.97 ^{aD}	97.41 ± 5.31 ^{fghD}	98.67 ± 7.84 ^{efD}	61.12 ± 8.59 ^{deC}	39.93 ± 4.12 ^{BB}	24.55 ± 4.57 ^{bA}
	LR-RE	100 ± 5.40 ^{aD}	61.93 ± 5.31 ^{bcC}	57.71 ± 1.18 ^{bcC}	51.92 ± 7.34 ^{cdBC}	44.47 ± 6.44 ^{BB}	24.98 ± 1.43 ^{bA}
<i>S. Typhimurium</i>	RE ¹	100 ± 11.92 ^{AB}	90.67 ± 4.64 ^{efgB}	97.37 ± 3.48 ^{efB}	65.15 ± 5.08 ^{defA}	64.20 ± 5.08 ^{CA}	59.28 ± 5.14 ^{dA}
	LP-RE	100 ± 3.56 ^{aC}	82.57 ± 11.48 ^{defB}	72.86 ± 1.53 ^{dB}	26.85 ± 6.36 ^{aA}	35.54 ± 2.62 ^{bA}	32.61 ± 5.58 ^{bcA}
	LR-RE	100 ± 3.42 ^{aD}	68.21 ± 4.80 ^{cdB}	61.89 ± 6.46 ^{CB}	74.11 ± 9.40 ^{efB}	37.01 ± 5.62 ^{bA}	35.49 ± 11.30 ^{bcA}
<i>E. coli</i> O157:H7	RE ¹	100 ± 6.67 ^{aB}	77.77 ± 3.61 ^{deA}	92.64 ± 4.87 ^{eB}	77.85 ± 3.92 ^{fA}	121.98 ± 5.59 ^{eC}	114.34 ± 7.00 ^{fC}
	LP-RE	100 ± 5.15 ^{aAB}	110.06 ± 3.12 ^{hB}	109.36 ± 2.88 ^{gAB}	110.86 ± 4.13 ^{gBA}	98.74 ± 7.87 ^{dA}	101.26 ± 2.99 ^{eAB}
	LR-RE	100 ± 3.73 ^a	101.60 ± 9.73 ^{ghA}	105.59 ± 3.84 ^{fgAB}	122.13 ± 10.57 ^{gBC}	123.24 ± 12.75 ^{eBC}	137.98 ± 8.54 ^{gC}
<i>S. aureus</i>	RE ¹	100 ± 4.36 ^{aD}	53.70 ± 6.82 ^{bb}	52.39 ± 2.01 ^{bb}	43.25 ± 4.56 ^{bcAB}	42.60 ± 5.31 ^{bAB}	36.35 ± 6.76 ^{bcA}
	LP-RE	100 ± 3.55 ^{aE}	97.43 ± 3.22 ^{fghE}	40.71 ± 4.36 ^{aD}	29.74 ± 2.59 ^{abC}	22.53 ± 1.43 ^{aB}	10.81 ± 1.98 ^{aA}
	LR-RE	100 ± 6.37 ^{aC}	32.02 ± 5.37 ^{aA}	32.26 ± 2.86 ^{aA}	42.13 ± 4.84 ^{bcAB}	40.12 ± 3.21 ^{bAB}	44.57 ± 3.20 ^{CB}

Uppercase letters (series "A-D) indicate significant (Duncan's range test, $p < 0.05$) differences in the same row, and lowercase letters (series "a-h") indicate significant (Duncan's range test, $p < 0.05$) differences in the same column

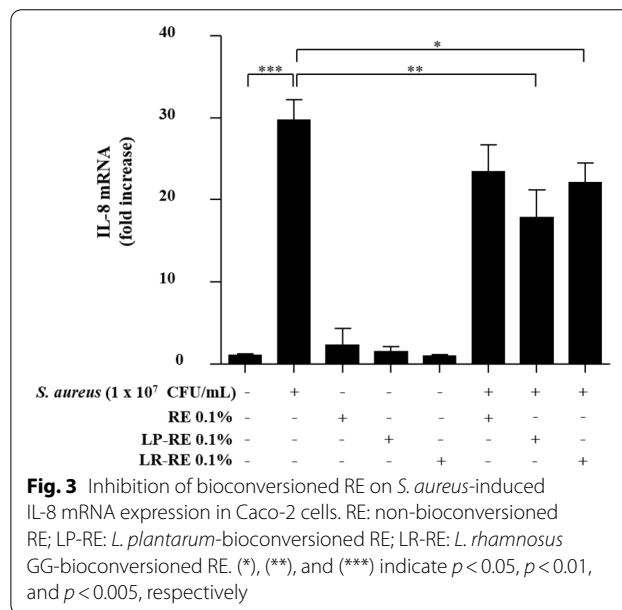
^a Results are expressed as average percentage ± standard deviation of biofilm (%)

^b Control is the biofilm formation of bacteria without sample and assigned to 100%



Tune software. The parameters for the full scan/dd-MS² mode were as follows: HESI-II voltages of 3500 V for the positive; S-lens radio frequency level of 50 V; heater temperature of 320 °C; and heated capillary temperature of 300 °C. Full-scan spectra were obtained over a mass range of 100–1500 Da. The MS and dd-MS² resolutions were set to 70,000 FWHM and 17,500 FWHM at $m/z=200$, respectively. The automatic gain control (AGC) was set to 10⁶ charges with a maximum injection time of 100 ms.

The Compound Discoverer software (Thermo Fisher Scientific, USA) was used for compound identification



based on the following parameters: (1) alignment parameter settings: mass tolerance, 5 ppm; maximum shift, 1 min. (2) Identification parameter settings: MS1 tolerance, 5 ppm; MS2 tolerance, 10 ppm; assignment threshold, 70%.

Statistical analysis

All experimental results were reported as the mean ± standard deviation of three independent replicate

Table 2 Univariate analysis of secondary metabolite profiles of not-bioconverted RE², *Lb. plantarum*-bioconverted RE³, *Lb. rhamnosus* GG-bioconverted RE (GG)

Compounds	Molecular formula	RT (min)	Sample peak area		
			RE ¹	LP-RE	LR-RE
L-Tyrosine	C ₉ H ₁₁ NO ₃	1.884	5.3 × 10 ⁸	5.0 × 10 ⁶	4.5 × 10 ⁶
L-Norleucine	C ₆ H ₁₃ NO ₂	2.131	2.6 × 10 ⁹	4.3 × 10 ⁷	2.2 × 10 ⁹
Isoleucine	C ₆ H ₁₃ NO ₂	1.964	4.2 × 10 ⁸	7.8 × 10 ⁶	1.5 × 10 ⁸
Indole-3-acrylic acid	C ₁₁ H ₉ NO ₂	6.36	3.3 × 10 ⁸	4.3 × 10 ⁶	2.0 × 10 ⁸
2'-O-Methyladenosine	C ₁₁ H ₁₅ N ₅ O ₄	2.253	3.4 × 10 ⁸	2.6 × 10 ⁸	1.4 × 10 ⁶
α-Aspartylphenylalanine	C ₁₃ H ₁₆ N ₂ O ₅	6.601	1.8 × 10 ⁷	2.1 × 10 ⁶	1.1 × 10 ⁶
Thymine	C ₅ H ₆ N ₂ O ₂	2.043	6.9 × 10 ⁷	2.1 × 10 ⁷	3.4 × 10 ⁷
L-Tryptophan	C ₁₁ H ₁₂ N ₂ O ₂	6.362	1.9 × 10 ⁸	2.1 × 10 ⁶	1.1 × 10 ⁸
L-Phenylalanine	C ₉ H ₁₁ NO ₂	3.348	1.7 × 10 ⁹	2.5 × 10 ⁷	1.4 × 10 ⁷
Kanosamine	C ₆ H ₁₃ NO ₅	1.429	1.7 × 10 ⁸	1.6 × 10 ⁶	1.1 × 10 ⁷
Hypoxanthine	C ₅ H ₄ N ₄ O	1.733	5.3 × 10 ⁸	4.9 × 10 ⁷	3.8 × 10 ⁷
Glycyl-L-leucine	C ₈ H ₁₆ N ₂ O ₃	2.607	1.2 × 10 ⁸	5.2 × 10 ⁵	5.1 × 10 ⁵
Glycerophospho-N-palmitoyl ethanolamine	C ₂₁ H ₄₄ NO ₇ P	21.194	5.9 × 10 ⁷	1.2 × 10 ⁶	2.3 × 10 ⁶
D,L-Arginine	C ₆ H ₁₄ N ₄ O ₂	1.175	5.7 × 10 ⁷	1.9 × 10 ⁷	7.2 × 10 ⁵
D-(+)-Pyroglutamic acid	C ₅ H ₇ NO ₃	1.79	1.1 × 10 ⁸	2.3 × 10 ⁸	2.4 × 10 ⁸
Cytosine	C ₄ H ₅ N ₃ O	1.263	2.0 × 10 ⁷	1.1 × 10 ⁸	6.1 × 10 ⁵
Agmatine	C ₅ H ₁₄ N ₄	1.162	4.6 × 10 ⁷	4.1 × 10 ⁶	3.5 × 10 ⁶
Adenosine	C ₁₀ H ₁₃ N ₅ O ₄	1.821	8.0 × 10 ⁸	3.5 × 10 ⁷	1.3 × 10 ⁷
2-Hydroxycinnamic acid	C ₉ H ₈ O ₃	1.877	2.2 × 10 ⁸	1.9 × 10 ⁶	1.3 × 10 ⁶
2'-Deoxyadenosine	C ₁₀ H ₁₃ N ₅ O ₃	1.868	1.6 × 10 ⁷	1.8 × 10 ⁶	1.9 × 10 ⁷
1-Methylguanine	C ₆ H ₇ N ₅ O	1.741	3.9 × 10 ⁷	7.3 × 10 ⁷	5.1 × 10 ⁷
Guanine	C ₅ H ₅ N ₅ O	1.729	1.9 × 10 ⁸	4.4 × 10 ⁸	6.4 × 10 ⁸
Nicotinic acid	C ₆ H ₅ NO ₂	1.731	1.3 × 10 ⁸	2.6 × 10 ⁸	3.5 × 10 ⁸
(±)12(13)-DiHOME	C ₁₈ H ₃₄ O ₄	18.275	4.2 × 10 ⁶	1.3 × 10 ⁷	7.3 × 10 ⁶
N6-Methyladenine	C ₆ H ₇ N ₅	1.784	2.6 × 10 ⁷	1.0 × 10 ⁸	1.1 × 10 ⁸
Indole-3-lactic acid	C ₁₁ H ₁₁ NO ₃	9.973	5.3 × 10 ⁶	1.0 × 10 ⁸	7.8 × 10 ⁷
Acetophenone	C ₈ H ₈ O	2.077	5.5 × 10 ⁶	6.7 × 10 ⁷	5.7 × 10 ⁷
9-Oxo-10(E),12(E)-octadecadienoic acid	C ₁₈ H ₃₀ O ₃	21.92	1.5 × 10 ⁶	4.9 × 10 ⁶	1.0 × 10 ⁷
4-Guanidinobutyric acid	C ₅ H ₁₁ N ₃ O ₂	1.315	9.5 × 10 ⁷	6.5 × 10 ⁷	7.4 × 10 ⁷
Pyridoxine	C ₈ H ₁₁ NO ₃	1.797	2.0 × 10 ⁷	2.8 × 10 ⁷	2.9 × 10 ⁷
Hexadecanamide	C ₁₆ H ₃₃ NO	25.682	1.0 × 10 ⁸	9.0 × 10 ⁷	1.2 × 10 ⁸
Choline	C ₅ H ₁₃ NO	1.241	1.9 × 10 ⁹	1.1 × 10 ⁹	1.5 × 10 ⁹
9-Oxo-ODE	C ₁₈ H ₃₀ O ₃	13.907	6.7 × 10 ⁷	1.1 × 10 ⁸	8.2 × 10 ⁷
Prostaglandin A2	C ₂₀ H ₃₀ O ₄	13.983	3.0 × 10 ⁶	3.0 × 10 ⁶	3.0 × 10 ⁶
Pipecolic acid	C ₆ H ₁₁ NO ₂	1.323	1.3 × 10 ⁷	5.8 × 10 ⁶	1.7 × 10 ⁷
Ornithine	C ₅ H ₁₂ N ₂ O ₂	1.149	9.1 × 10 ⁶	2.5 × 10 ⁶	1.1 × 10 ⁷
Nootkatone	C ₁₅ H ₂₂ O	21.729	2.1 × 10 ⁷	2.1 × 10 ⁷	2.5 × 10 ⁷
N6,N6,N6-Trimethyl-L-lysine	C ₉ H ₂₀ N ₂ O ₂	1.172	1.3 × 10 ⁷	1.2 × 10 ⁷	1.5 × 10 ⁷
N,N-Dimethylsphingosine	C ₂₀ H ₄₁ NO ₂	21.278	2.8 × 10 ⁶	2.8 × 10 ⁶	4.0 × 10 ⁶
Mono(2-ethylhexyl) phthalate (MEHP)	C ₁₆ H ₂₂ O ₄	22.144	3.3 × 10 ⁷	4.6 × 10 ⁷	4.1 × 10 ⁷
L-Histidine	C ₆ H ₉ N ₃ O ₂	1.156	1.7 × 10 ⁷	1.2 × 10 ⁶	1.7 × 10 ⁷
L-Glutathione (reduced)	C ₁₀ H ₁₇ N ₃ O ₆ S	1.727	4.0 × 10 ⁵	2.5 × 10 ⁵	5.0 × 10 ⁵
Indole-3-acetic acid	C ₁₀ H ₉ NO ₂	11.13	7.9 × 10 ⁶	1.0 × 10 ⁷	1.2 × 10 ⁷
Betaine	C ₅ H ₁₁ NO ₂	1.293	1.5 × 10 ⁸	9.3 × 10 ⁷	2.5 × 10 ⁸
2-Amino-1,3,4-octadecanetriol	C ₁₈ H ₃₉ NO ₃	17.473	4.6 × 10 ⁶	4.4 × 10 ⁶	4.6 × 10 ⁶
15-epi Prostaglandin A1	C ₂₀ H ₃₂ O ₄	12.644	1.8 × 10 ⁷	1.8 × 10 ⁷	1.8 × 10 ⁷

experiments. Statistical differences between the treatment groups and the appropriate controls were determined via the Student's t-test using GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA) or one-way analysis of variance [12] using IBM SPSS Statistics 23 (IBM, Armonk, NY, USA).

Results and discussion

Anti-bacterial effects

Anti-bacterial activity of bioconverted RE

Our study determined the inhibitory effects of LP-RE and LR-RE on the growth of *Y. enterocolitica*, *S. Typhimurium*, *E. coli* O157:H7, and *S. aureus*. As illustrated in Fig. 1A, the growth of *Y. enterocolitica* was effectively suppressed when the bacteria were incubated with both LP-RE and LR-RE. The bioconverted RE inhibited the growth of *Y. enterocolitica* more effectively than RE. LP-RE and LR-RE did not significantly inhibit the growth of *Y. enterocolitica* from 0 to 24 h of incubation; however, significant growth suppression effects were observed after 48 h of incubation. Furthermore, LP-RE and LR-RE suppressed the growth of *S. Typhimurium* at 24 and 48 h, whereas the RE treatment increased *S. Typhimurium* growth (Fig. 1B). In contrast, neither LP-RE nor LR-RE inhibited the growth of *E. coli* O157:H7 (Fig. 1C). RE significantly inhibited *S. aureus* growth from 6 to 24 h but did not inhibit bacterial growth at 48 h. Furthermore, only LR-RE (not RE nor LP-RE) suppressed *S. aureus* growth at 48 h (Fig. 1D). Several studies have shown that antimicrobial activity can be enhanced via biotransformation. Enhancement of antimicrobial activity via biotransformation has been constantly reported such as biotransformed Chinese herbal medicine residue with *Aspergillus oryzae* showed increased antibacterial activity to pathogenic bacteria [13], and bioconversion of fruit juice using LAB has an anti-listerial effect [14]. Therefore, the increase in antimicrobial activity by bioconversion of rice extract supports that the research result increases the antimicrobial activity of food by fermentation.

Bioconverted RE inhibits bacterial biofilm formation

To examine whether bioconverted RE inhibits pathogenic bacteria biofilm formation, *Y. enterocolitica*, *S. Typhimurium*, *E. coli* O157:H7, and *S. aureus* were incubated with or without bioconverted RE (0%, 3.125%, 6.25%, 12.5%, 25%, and 50%) for 24 h. As shown in Table 1, both the bioconverted and control RE significantly inhibited *Y. enterocolitica* biofilm formation in a dose-dependent manner. At the highest concentration (50%), RE substantially inhibited *S. Typhimurium* biofilm formation ($59.28 \pm 5.14\%$). However, both LP-RE and LR-RE decreased biofilm formation more effectively

($32.61 \pm 5.58\%$ and $35.49 \pm 11.30\%$, respectively). Similar to the inhibition pattern of bacterial growth, *E. coli* O157:H7 biofilm formation was not inhibited in the presence of either LP-RE or LR-RE. *S. aureus* biofilm formation appeared to be effectively inhibited by LP-RE and LR-RE, as well as RE. However, except for the 3.125% LP-RE treatment, both LP-RE and LR-RE inhibited *S. aureus* biofilm formation more significantly. Particularly, 50% of LP-PE strongly suppressed *S. aureus* biofilm formation ($10.81 \pm 1.98\%$ of biofilm formation). These results suggest that the bioconverted RE possessed potent anti-biofilm activities against *Y. enterocolitica*, *S. Typhimurium*, and *S. aureus*, but not against *E. coli* O157:H7. Inhibition effect of biofilm formation is caused by inhibiting the growth of pathogen or inhibition of biofilm formation [15]. In our growth inhibition result, except for *E. coli*, growth inhibition was the effect. Therefore, the anti-biofilm activity of bioconversion rice extract could be deemed to suppress of growth of the pathogen.

Anti-inflammatory effects

Effect of bioconverted RE on the viability of Caco-2 cells

Cytotoxicity must be thoroughly tested to determine the suitability, applicability, and safety of food extracts. Therefore, the MTT assay was conducted to confirm the viability of Caco-2 cells in the presence or absence of bioconverted RE (10%, v/v).

Figure 2 illustrates the cell viability results of the bioconverted RE after 24 h of incubation. Notably, cell viability was not affected by RE, LP-RE, and LR-RE treatment regardless of concentration, suggesting that bioconverted RE is not cytotoxic toward Caco-2 cells.

Bioconverted RE inhibits *S. aureus*-induced IL-8 expression

Consistent with our antibacterial and anti-biofilm results, bioconversion RE was affected on *S. aureus*. Therefore we demonstrated to investigate whether bioconverted RE could inhibit *S. aureus*-induced IL-8 expression, Caco-2 cells were treated with 0.1% LP-RE, LR-RE, or RE in the presence or absence of *S. aureus* (1×10^7 CFU/mL) for 3 h. As illustrated in Fig. 3, LP-RE, LR-RE, and RE did not increase IL-8 mRNA expression, whereas *S. aureus* significantly increased IL-8 mRNA expression in Caco-2 cells. When the cells were co-incubated with bioconverted RE and *S. aureus*, IL-8 mRNA expression was significantly decreased in the presence of LP-RE and LR-RE. Although RE moderately inhibited *S. aureus*-induced IL-8 mRNA expression, both LP-RE and LR-RE more effectively inhibited *S. aureus*-induced IL-8 mRNA expression, suggesting that the bioconverted RE

has anti-inflammatory effects in human intestinal epithelial cells. In previous studies, the anti-inflammatory effect was caused by the increment of metabolites and enhanced bioaccessibility or bioavailability of phenolic compounds [16–18].

Analysis of untargeted secondary metabolites of bioconverted RE by LC–MS/MS

Table 2 summarises the results of secondary metabolite analysis in RE using HPLC–MS/MS (Additional file 1: Fig. S1). Compared with the control, the concentration of 13 compounds increased significantly after bioconversion. Among these compounds, four (acetophenone, 9-oxo-10(E), 12(E)-octadecadienoic acid, nicotinic acid, and indol-3-lactic acid) were found to enhance the therapeutic effects of the extract. Particularly, acetophenone and 9-oxo-10(E), 12(E)-octadecadienoic acid have been previously reported to increase anti-bacterial and anti-inflammatory activity [10, 11, 19]. Furthermore, nicotinic acid and L-indole-3-lactic acid exhibit anti-inflammatory effects and radical scavenging activity, respectively [20, 21]. After the bioconversion process, the levels of many types of amino acids decreased and lactic acid bacteria likely used these free amino acids to synthesise the above-mentioned compounds.

Conclusions

Collectively, our findings suggest that lactic acid bacteria-bioconverted RE can suppress *S. Typhimurium*, *Y. enterocolitica*, and *S. aureus* growth and biofilm formation more effectively than RE; however, *E. coli* O157:H7 remained largely unaffected. Additionally, this study demonstrated that LP-RE inhibited interleukin-8 secretion by *S. aureus* in the human intestinal epithelial cell line Caco-2. Moreover, our study demonstrated that LC/MS–MS can be used to monitor the production of a variety of secondary metabolites produced during bioconversion. More importantly, these secondary metabolites were linked to antibacterial and anti-inflammatory effects, suggesting that bioconversion is a promising means to produce dietary supplements with therapeutic properties. Nevertheless, additional studies are required to confirm the efficacy of bioconverted RE as a health functional food, particularly when consumed daily.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40538-022-00311-5>.

Additional file 1: Figure S1. Typical LC–MS chromatogram of untargeted secondary metabolites of bioconverted RE.

Acknowledgements

Not applicable.

Author contributions

H-JK: formal analysis, investigation, methodology; HA: formal analysis; BSK: methodology; S-SK: methodology; K-GL: supervision; validation; investigation; project administration. All authors read and approved the final manuscript.

Funding

This study was supported by the Basic Science Research Program through the National Research Foundation of Korea [grant number NRF, 2021R1A2B5B01002296].

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 21 February 2022 Accepted: 14 June 2022

Published online: 27 June 2022

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