Microbiol. Biotechnol. Lett. (2017), 45(1), 35–42
http://dx.doi.org/10.4014/mbl.1701.01002
pISSN 1598-642X eISSN 2234-7305

MBL
Microbiology and Biotechnology Letters

# Enhancement of Skin Antioxidant and Anti-Inflammatory Potentials of *Agastache rugosa* Leaf Extract by Probiotic Bacterial Fermentation in Human Epidermal Keratinocytes

Hye-Won Lim[1†], Yoonjin Lee[2†], Yu-Hua Huang[1], Ji-Young Yoon[3], Su Hee Lee[4], Kyunghoon Kim[4*], and Chang-Jin Lim[1,5*]

[1]*Shebah Biotech Inc., G-Tech Village, Chuncheon 24398, Republic of Korea*
[2]*R&D Center, Cosmocos Corporation, Incheon 21698, Republic of Korea*
[3]*Jeonju AgroBio-Materials Institute, Joenju 54810, Republic of Korea*
[4]*Department of Biological Sciences,* [5]*Department of Biochemistry, Kangwon National University, Chuncheon 24341, Republic of Korea*

Received: February 1, 2017 / Revised: March 23, 2017 / Accepted: March 24, 2017

**This study aimed to investigate the effects of probiotic fermentation by comparing the skin antioxidant and anti-inflammatory properties of non-fermented (ARE) and fermented (ARE-F) hot water extracts of *Agastache rugosa* leaves. ARE-F was obtained via ARE fermentation using *Lactobacillus rhamnosus* HK-9. In vitro, anti-inflammatory properties were evaluated by analyzing the levels of nitric oxide (NO), reactive oxygen species (ROS), and inducible nitric oxide synthase (iNOS) in lipopolysaccharide (LPS)-stimulated HaCaT keratinocytes. In vitro antiradical activity was measured using 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. Attenuation of LPS-stimulated NO ($p < 0.01$), ROS ($p < 0.001$) and iNOS ($p < 0.05$) levels by ARE-F was significantly stronger than that by ARE in HaCaT keratinocytes. However, no differences were observed between the DPPH radical scavenging activities of ARE and ARE-F. ARE-F possesses enhanced skin antioxidant and anti-inflammatory properties, suggesting that probiotic bacterial fermentation can be considered an effective tool for augmenting some pharmacological properties of *A. rugosa* leaves. In brief, the skin antioxidant and anti-inflammatory potentials of *A. rugosa* leaf extract are augmented by the fermentation with *L. rhamnosus* HK-9, a probiotic bacterium.**

**Keywords:** ***Agastache rugosa,*** **inducible nitric oxide synthase, nitric oxide, reactive oxygen species, anti-inflammatory, antiradical**

## Introduction

*Agastache rugosa* Kuntze, a perennial herb of the mint family (*Lamiaceae*), is grown in East Asian Countries, including Korea, China and Japan. In traditional folk medicine, it has been used to treat colds, anorexia, cholera, vomiting, miasma and other kinds of disorders [1]. Various essential oils and a variety of flavonoids, such as acacetin-7-*O*-β-D-glucopyranoside (tilianin), acacetin, linarin, agastachoside and rosmarinic acid, have been isolated from the different parts of *A. rugosa* [2]. Various *A. rugosa* extracts and ingredients exhibit a range of pharmacological activities, such as antimicrobial, antifungal, insecticidal, antiviral, antihypertensive, anti-inflammatory, anticancer, antioxidative, antiathero-

***Corresponding authors**
K. K.
Tel: +82-33-250-8527, Fax: +82-33-259-5665
E-mail: kkim@kangwon.ac.kr
C.-J. L.
Tel: +82-33-256-6090, Fax: +82-303-0957-6090
E-mail: cjlim@kangwon.ac.kr
†These authors contributed equally to this work.

genic and vasorelaxant activities [3–6]. However, the dermatological benefits of *A. rugosa* have yet to be evaluated.

Probiotic bacterial fermentation, emerging as a crucial processing method in cosmetic technologies, can diminish the toxicities of cosmetic resources, enhance the absorption into human skin by altering their molecular structures, and improve their desirable cosmetic activities [7]. It can also play a key role in developing cosmeceuticals which are cosmetic products containing pharmacologically active ingredients purporting to have therapeutic benefits. Cosmeceutical ingredients typically possess useful properties such as antioxidant, anti-inflammatory, skin whitening, anti-photoaging, anti-wrinkle or photoprotective activity [8].

Antioxidative and antisenescent activities of an aqueous extract of *Acanthopanax koreanum* were augmented by fermentation with lactic acid bacteria, *Lactobacillus plantarum* and *Bifidobactrium bifidum* [7]. Antioxidative activities of soybean products, such as miso, tempeh and natto, fermented with *Aspergillus oryzae*, *Rhizopus oligosporum* and *Bacillus natto* are significantly higher than those of non-fermented steamed soybean [9]. Antioxidative and skin whitening activities of soybean extract fermented with *Bacillus subtilis* are also higher than those of non-fermented soybean extract [10]. Fermentation of cultured wild ginseng root and red ginseng extracts with probiotic *Bifidobacterium longum* strengthens their antioxidative and anti-inflammatory activities [11,12]. These findings imply that probiotic fermentation can offer various advantages in the application of natural products, including the development of functional cosmetic resources.

In this work, we tried to assess the effects of probiotic bacterial fermentation on the skin antioxidant and anti-inflammatory activities of a hot water extract of *A. rugosa* leaves in human epidermal keratinocytes. This work proposes a new possibility that *Lactobacillus rhamnosus*-fermented *A. rugosa* extract can be utilized as a valuable cosmetic resource.

## Materials and Methods

### Chemicals

Ascorbic acid, bovine serum albumin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Bradford reagent, 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA), lipopolysaccharide (LPS), sodium nitrite, and Griess reagent were from Sigma-Aldrich Chemical Co. (USA). Cell lysis buffer [25 mM Tris-phosphate (pH 7.8), 2 mM 1,2-diaminocyclohexane-*N*,*N*,*Nv*,*Nv*-tetraactic acid, 2 mM dithiothreitol, 10% glycerol, 1% Triton X-100] was obtained from Promega Korea (Korea).

### Plant material

Dried *A. rugosa* leaves, obtained at a local market, Chuncheon, Korea, in September 2015, were authenticated by Prof. Ki-Oug Yoo, Department of Biological Sciences, Kangwon National University, Chuncheon, Korea. The voucher specimen of the plant material was deposited in the herbarium of the same department under the acquisition number KWNU90446.

### Preparation of non-fermented (ARE) and fermented (ARE-F) hot water extracts

Dried *A. rugosa* leaves, ground under liquid nitrogen, were mixed with 10-fold distilled water in 2-liter conical flask and extracted under reflux by placing in a water bath at 90℃ for 4 h. After being filtered through a filter paper, the hot water extract was evaporated to dryness in a freeze dryer, and the extract powder was named ARE. The yield was determined to be 10.4%.

ARE, after being resuspended in distilled water, was incubated at 30℃ for 5 days with a culture of *L. rhamnosus* HK-9 (KACC 11254P, Korea), a laboratory-stocked strain of Shebah Biotech Inc., centrifuged at 5,000 × *g* for 20 min to discard bacterial cells, and concentrated using a freeze dryer to produce fermented extract powder, named ARE-F.

For the experiments, ARE and ARE-F were dissolved in dimethyl sulfoxide, and control cells were subjected to vehicle only (0.1% dimethyl sulfoxide).

### Cell culture

An immortalized HaCaT keratinocyte cell line (ATCC, USA) was grown in DMEM containing 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin in a humidified atmosphere with 5% $CO_2$ at 37℃.

### Preparation of cellular lysate

Adherent cells, washed twice with phosphate-buffered

http://dx.doi.org/10.4014/mbl.1701.01002

saline (PBS) and kept on ice for 5 min, were obtained by scraping cells from the dish with a cell scraper and centrifuged at 5,000 × *g* for 10 min. Cell pellets were resuspended in cell lysis buffer and kept for 30 min on ice. Cellular lysate was taken after centrifugation at 5,000 × *g* for 15 min.

The protein content of cellular lysates was determined according to the Bradford protein assay [13] using bovine serum albumin as the protein standard.

#### Quantitation of nitrite in conditioned medium

Accumulated nitrite ($NO_2^-$), an index of cell-released NO, in conditioned media was quantitated using a spectrophotometric assay based upon Griess reaction [14]. In brief, an equal volume of Griess reagent (1% sulfanilamide - 0.1% *N*-1-naphthyl-ethylenediamine dihydrochloride in 2.5% phosphoric acid) was incubated with conditioned medium for 10 min at room temperature, and then the absorbance at 550 nm was measured using a microplate reader (SpectraMax iD3 Multi-Mode, Molecular Devices, USA). The calibration curve was constructed using known concentrations (0–160 μM) of sodium nitrite.

#### Quantitation of intracellular ROS

A fluorescent ROS probe DCFH-DA, which produces the fluorescent 2',7'-dichlorofluorescein (DCF; $\lambda_{excitation}$ = 485 nm, $\lambda_{emission}$ = 530 nm) upon enzymatic reduction and oxidation by ROS, was used [15]. After preincubation with varying concentrations of ARE or ARE-F for 1 h, the cells were treated for 24 h with 1 μg/ml LPS, if necessary. The cells were incubated with 5 μM DCFH-DA for 30 min at 37℃ and harvested. They were twice washed with 1 ml FBS-free medium and resuspended in 1 ml FBS-free medium. The intracellular ROS levels were determined by monitoring the fluorescence using Multi-Mode Microplate Reader (Synergy™ Mx, BioTek Instruments, USA).

#### Western blotting analysis

Western blotting analysis was performed to detect iNOS in cellular lysate using anti-iNOS antibody (610332, BD Transduction Laboratories, USA). GAPDH, used as an internal loading standard, was detected using anti-GAPDH antibody (LF-PA0212, AbFrontier, Korea). After the membrane was probed with primary antibodies overnight at 4℃, it was incubated with secondary antibody (goat anti-rabbit IgG-pAb-HRP-conjugate; ADI-SAB-300, Enzo Life Sciences, USA) for 1 h at room temperature, and developed using an enhanced Westsave up™ (AbFrontier, Korea).

#### DPPH radical scavenging activity assay

The DPPH radical scavenging activities of ARE and ARE-F were determined according to the method described earlier [16] with some modifications. In brief, the reaction mixtures containing 30 μl of varying concentrations of ARE or ARE-F and 270 μl of 0.1 mM DPPH in a 96-well microtiter plate were kept in the dark at room temperature for 30 min, and the absorbance was measured at 517 nm. Ascorbic acid was used as a positive control.

$$\text{DPPH radical scavenging activity (\%)} = [(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100$$

#### Statistical analysis

The results were represented as mean ± standard deviation (SD). Differences between experimental groups were analyzed using one-way ANOVA followed by post hoc Tukey HSD test for multiple comparisons. A $p$ value less than 0.05 was considered statistically significant.

## Results

#### Suppression of the LPS-stimulated NO production

Since NO, an intracellular pro-inflammatory mediator, reacts with superoxide radical to generate a peroxynitrite ion leading to a variety of inflammatory states, the suppression of NO production is closely linked with an anti-inflammatory action [17]. When HaCaT keratinocytes were stimulated with LPS, a potent microbial initiator of inflammation, the nitrite content, as an index of NO, was increased about 3.2-fold (Fig. 1). When HaCaT keratinocytes were pretreated with ARE at 5, 20 and 80 μg/ml, the nitrite content was attenuated to 93.1, 80.6 and 44.4% of that of the LPS only, respectively (Fig. 1A). As shown in Fig. 1B, ARE-F, at the concentrations of 5, 20 and 80 μg/ml, could attenuate the nitrite content to 46.4, 23.2 and 4.3%, respectively, over that of the LPS only. The $IC_{50}$ values of ARE and ARE-F was 68.1 and 7.9 μg/ml, respectively. The

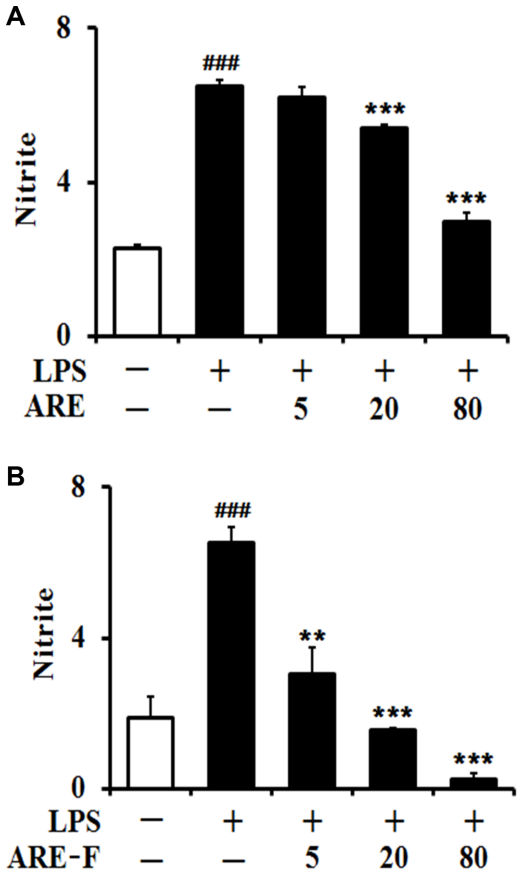


**Fig. 1. Suppressive effects of non-fermented (ARE, A) and fermented (ARE-F, B) hot water extracts of *Agastache rugosa* leaves on the LPS-stimulated elevation of nitric oxide (NO) in HaCaT keratinocytes.** After HaCaT cells were subjected to the varying concentrations (0, 5, 20 and 80 μg/ml) of ARE (A) and ARE-F (B) for 1 h, the cells were treated with 1 μg/ml LPS for 24 h. The accumulated nitrite, an index of NO, in conditioned medium was determined based upon Griess reaction. Each bar shows the mean ± SD of three independent experiments repeated in triplicate. $^{###}p < 0.001$ vs. the non-treated control. $^{**}p < 0.01$; $^{***}p < 0.001$ vs the LPS only.

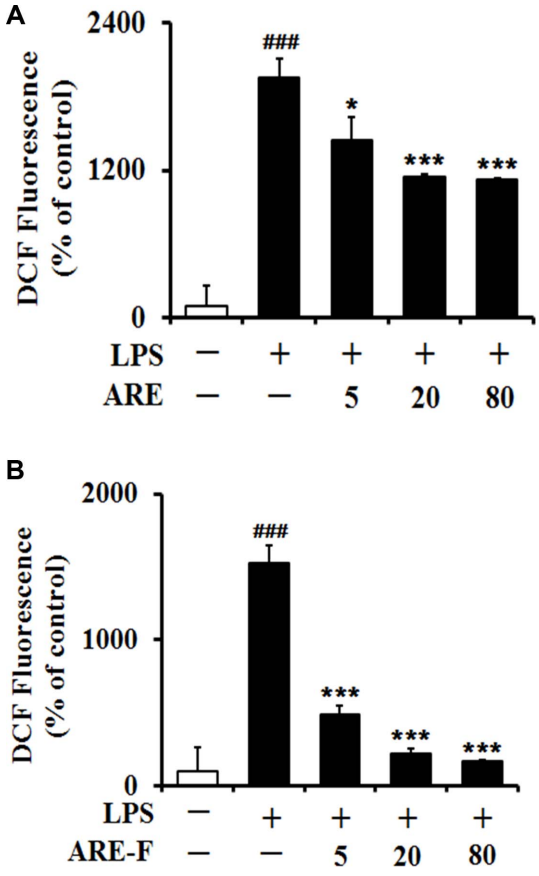


**Fig. 2. Suppressive effects of non-fermented (ARE, A) and fermented (ARE-F, B) hot water extracts of *Agastache rugosa* leaves on the LPS-stimulated reactive oxygen species (ROS) elevation in HaCaT keratinocytes.** After HaCaT cells were subjected to the varying concentrations (0, 5, 20 and 80 μg/ml) of ARE (A) and ARE-F (B) for 1 h, the cells were treated with 1 μg/ml LPS for 24 h. The ROS level is represented as relative DCF fluorescence, expressed as a percentage (%) of the corresponding non-treated control. Each bar shows the mean ± SD of three independent experiments repeated in triplicate. $^{###}p < 0.001$ vs. the non-treated control. $^{*}p < 0.05$; $^{***}p < 0.001$ vs the LPS only.

vehicle used in this work had no effect on cell viability. Collectively, probiotic fermentation is capable of enhancing the suppressive activity of ARE on the LPS-stimulated NO production in HaCaT keratinocytes.

### Suppression on the LPS-stimulated ROS production

ROS, known as key signaling molecules that play a critical role in inflammatory process, promote chronic inflammation through the induction of cyclooxygenase-2, inflammatory cytokines (TNF-α, interleukin-1, interleukin-6), chemokines (interleukin-8, C-X-C chemokine receptor type 4) and pro-inflammatory transcription factors [18]. In HaCaT keratinocytes, the ROS levels were enhanced about 18.5-fold by the LPS treatment (Fig. 2). The elevated ROS levels were attenuated to 71.0, 59.4 and 57.9% over that of the LPS only by ARE at 5, 20 and 80 μg/ml, respectively (Fig. 2A). ARE-F at 5, 20 and 80 μg/ml could attenuate the LPS-stimulated ROS levels to 31.3, 14.1 and 10.9%, respectively (Fig. 2B). The $IC_{50}$ values of ARE and ARE-F were 96.7 and 4.3 μg/ml, respectively. Taken together, the suppressive activity of

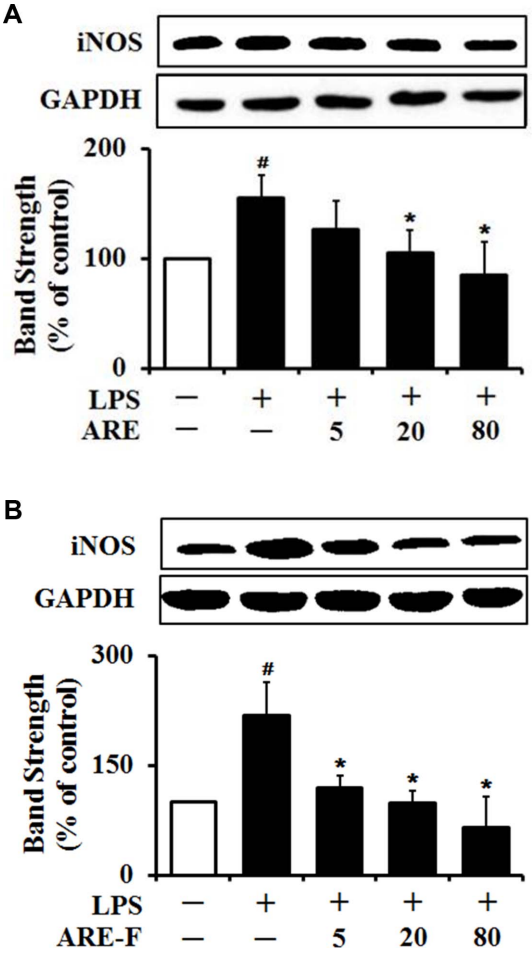


**Fig. 3. Suppressive effects of non-fermented (ARE, A) and fermented (ARE-F, B) hot water extracts of *Agastache rugosa* leaves on the LPS-stimulated-inducible nitric oxide synthase (iNOS) production in HaCaT keratinocytes.** After HaCaT cells were subjected to the varying concentrations (0, 5, 20 and 80 μg/ml) of ARE (A) and ARE-F (B) for 1 h, the cells were treated with 1 μg/ml LPS for 24 h. iNOS in cellular lysates was detected using western blottting analysis. GAPDH was used as a protein loading control. In the lower panels, the relative band strength, expressed as a percentage (%) of the non-treated control, was determined with densitometry using the ImageJ software which can be downloaded from the NIH website. $^{\#}p < 0.05$ vs. the non-treated control. $^{*}p < 0.05$ vs. the LPS only.

ARE on the LPS-stimulated ROS levels can be markedly enhanced by probiotic bacterial fermentation in HaCaT keratinocytes.

### Down-regulation of LPS-stimulated iNOS production

Effects of ARE and ARE-F on the enhanced production of iNOS in the LPS-stimulated HaCaT keratinocytes

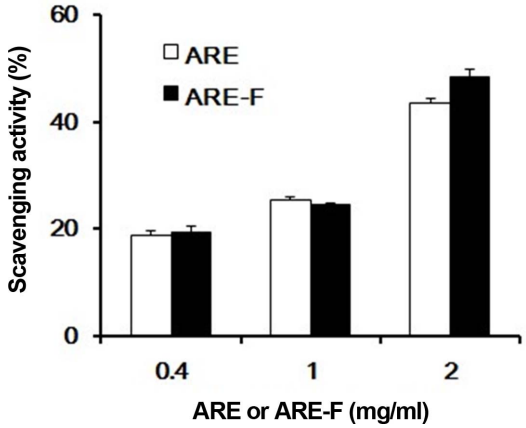


**Fig. 4. The DPPH radical scavenging activities of non-fermented (ARE, A) and fermented (ARE-F, B) hot water extracts of *Agastache rugosa* leaves.** The DPPH radical scavenging activities of ARE and ARE-F were determined using three different concentrations, such as 0.4, 1.0 and 2.0 mg/ml. The half-maximal scavenging concentration ($SC_{50}$) of ascorbic acid, used as a positive control, was determined to be 0.05 mg/ml.

were evaluated using western blotting analysis. LPS enhanced the iNOS protein levels to 1.7-fold in HaCaT keratinocytes (Fig. 3). ARE, at 5, 20 and 80 μg/ml, was able to diminish the LPS-induced iNOS production to 80.0, 68.0 and 54.0%, respectively, giving rise to an $IC_{50}$ value of 110.9 μg/ml (Fig. 3A). However, ARE-F, at 5, 20 and 80 μg/ml, could attenuate the LPS-induced iNOS production to 56.5, 45.7 and 30.4%, respectively, giving an $IC_{50}$ value of 30.0 μg/ml (Fig. 3B). This result confirms that ARE has an attenuating activity on the NO elevation via down-regulation of the LPS-stimulated iNOS production in HaCaT keratinocytes. In brief, the attenuating activity of ARE on the elevation of iNOS production in HaCaT keratinocytes is enhanced by probiotic bacterial fermentation.

### In vitro antiradical activity

The in vitro antioxidant potential of ARE and ARE-F were compared using DPPH method at their varying concentrations, 0.4, 1.0 and 2.0 mg/ml (Fig. 4). Ascorbic acid, used as a positive control, exhibited the half-maximal scavenging concentration ($SC_{50}$) of 0.05 mg/ml. As shown in Fig. 4, both ARE and ARE-F, at the concentrations tested, exhibited the similar levels of DPPH radical scavenging activities. This result implies that the antiradical activity of ARE was not affected by the fermenta-

tion with *L. rhamnosus*.

## Discussion

Until recently, chemical composition and pharmacological properties of *A. rugosa* have been continuously studied in more detail. Essential oils of *A. rugosa* flowers and leaves possess antimicrobial, antibiofilm and antitumor activities [19]. Among a total of 18 polyphenols identified from the flowers, leaves, stems and roots of *A. rugosa*, hydroxycinnamic acid is the most abundant compound in the roots, while the flowers contain the highest total flavonoid levels [20]. Antioxidant activities of *A. rugosa* is the highest in the stems, followed by leaves, flowers and roots in descending order [20]. An *A. rugosa* leaf extract enhances heme oxygenase-1 activity and protein via protein kinase G signaling pathway, and protects RAW264.7 macrophage cells form hydrogen peroxide-induced cytotoxicity [21]. An *A. rugosa* leaf extract diminishes the expression of iNOS protein and the NO production in rat osteosarcoma cells activated with a mixture of inflammatory cytokines including TNF-α and IL-1β via the prevention of the activation and translocation of NF-κB p65 to the nucleus [22]. It is demonstrated, in this work, that both non-fermented and fermented hot water extracts of *A. rugosa* leaves have skin anti-inflammatory activities via attenuating the NO, ROS and iNOS levels enhanced by LPS. These findings were obtained using human epidermal keratinocytes in order to focus on the skin anti-inflammatory activity of *A. rugosa* leaves. The skin anti-inflammatory activity of *A. rugosa* leaves might suggest their plausible application in the manufacture of anti-photoaging cosmetics.

Since fermented cosmetics, typically natural ingredients that are fermented, are usually more potent but have less adverse side effects than the non-fermented counterparts, they are considered to be better suited for sensitive and dry skin, and more symbiotic with the skin due to mimicking the skin's cell functions and supporting the skin without disrupting its natural process [8]. In addition to the enhancement of antioxidative and anti-inflammatory activities of cosmetic resources, probiotic fermentation has been diversely applied to the area of cosmetic industry. Fermentation of red ginseng with *Lactobacillus brevis* increases the contents of ginsenoside metabolites, such as Rg3, Rg5, Rk1, compound K, Rh1, F2, Rg2 and flavonoids content, and offers enhanced anti-wrinkle and whitening efficacies and reduced toxicological potency, compared to non-fermented red ginseng [23]. Fermentation with *Alcaligenes piechaudii* CC-ESB2 could augment the tyrosinase inhibitory and antioxidative activities of *Rhodiola rosea* and *Lonicera japonica* [24]. Based upon probiotic technology utilizing natural beneficial bacteria commonly found in healthy mouths to provide a natural defense against those bacteria harmful to teeth and gums, the probiotic mouth rinse was found to be effective in reducing plaque accumulation and gingival inflammation [25]. An aqueous fraction of *Cinnamomum cassia*, produced by solid state fermentation with *Phellinus baumi*, has the potential to alleviate the symptoms of atopic dermatitis in a mouse model of 2,4-dinitrofluorobenzene-induced atopic dermatitis [26]. Bifidobacterium-fermented soymilk extract, but not non-fermented soymilk extract, enhances the production of hyaluronic acid in monolayer and organotypic cultures of human keratinocytes, in cultures of human skin fibroblasts, and in hairless mouse skin, mainly due to genistein released from its glycoside during fermentation [27]. A *Bacillus subtilis natto*-fermented extract of *Radix astragali*, but not a corresponding non-fermented extract, also stimulates hyaluronic acid production in both cultured primary human epidermal keratinocytes and dermal fibroblasts, suggesting its role in preventing the age-dependent loss of hyaluronic acid content in aged human skin [28]. This work proves that *L. rhamnosus* can ferment a hot water extract of *A. rugosa* leaves to significantly enhance its skin anti-inflammatory activity. Although the underlying enhancing mechanism(s) remain to be solved, one probable mechanism might be based upon the production of aglycones or other metabolites of flavonoids and other cosmetic ingredients during fermentation. Additionally, no differences between the in vitro antiradical activities of ARE and ARE-F might suggest the mediation of metabolites, generated during fermentation, which penetrate readily into epidermal keratinocytes. Correct mechanism(s) on the enhancement by the fermentation with *L. rhamnosus* in the anti-inflammatory activity of *A. rugosa* leaf extract require advanced approaches in the future.

In conclusion, the skin antioxidant and anti-inflam-

http://dx.doi.org/10.4014/mbl.1701.01002

matory properties of a hot water extract from *A. rugosa* leaves were ascertained using HaCaT keratinocytes. Those beneficial potentials of the *A. rugosa* extract on the skin were significantly augmented by the fermentation with *L. rhamnosus*. In brief, probiotic fermentation technology can be utilized to strengthen the desirable dermatological properties of *A. rugosa* and broaden its diverse applicability in the field of skin care resources.

## Acknowledgements

This research was supported by the Ministry of Trade, Industry & Energy (MOTIE), Korea Institute for Advancement of Technology (KIAT) through the Encouragement Program for the Industries of Economic Cooperation Region (R0005382).

## References

1. Kim YB, Kim JK, Uddin MR, Xu H, Park WT, Tuan PA, *et al*. 2013. Metabolomics analysis and biosynthesis of rosmarinic acid in *Agastache rugosa* Kuntze treated with methyl jasmonate. *PLoS One* **8**: e64199.
2. Li HQ, Liu QZ, Liu ZL, Du SS, Deng ZW. 2013. Chemical composition and nematicidal activity of essential oil of *Agastache rugosa* against *Meloidogyne incognita*. *Molecules* **18**: 4170-4180.
3. Hong JJ, Choi JH, Oh SR, Lee HK, Park JH, Lee KY, *et al*. 2001. Inhibition of cytokine-induced vascular cell adhesion molecule-1 expression; possible mechanism for anti-atherogenic effect of *Agastache rugosa*. *FEBS Lett*. **495**: 142-147.
4. Gao LP, Wei HL, Zhao HS, Xiao SY, Zheng RL. 2005. Antiapoptotic and antioxidant effects of rosmarinic acid in astrocytes. *Pharmazie* **60**: 62-65.
5. Swarup V, Ghosh J, Ghosh S, Saxena A, Basu A. 2007. Antiviral and anti-inflammatory effects of rosmarinic acid in an experimental murine model of Japanese encephalitis. *Antimicrob. Agents Chemother.* **51**: 3367-3370.
6. Tuan PA, Park WT, Xu H, Park NI, Park SU. 2012. Accumulation of tilianin and rosmarinic acid and expression of phenylpropanoid biosynthetic genes in *Agastache rugosa*. *J. Agric. Food Chem.* **60**: 5945-5951.
7. Park MJ, Bae YS. 2016. Fermented *Acanthopanax koreanum* root extract reduces UVB- and $H_2O_2$-induced senescence in human skin fibroblast cells. *J. Microbiol. Biotechnol.* **26**: 1224-1233.
8. Antonopoulou I, Varriale S, Topakas E, Rova U, Christakopoulos P, Faraco V. 2016. Enzymatic synthesis of bioactive compounds with high potential for cosmeceutical application. *Appl. Microbiol. Biotechnol.* **100**: 6519-6543.
9. Lin CH, Wei YT, Chou CC. 2006. Enhanced antioxidative activity of soybean koji prepared with various filamentous fungi. *Food Microbiol.* **23**: 628-633.
10. Chae GY, Ha BJ. 2011. The comparative evaluation of fermented and non-fermented soybean extract on antioxidation and whitening. *Toxicol. Res.* **27**: 205-209.
11. Jung HJ, Choi H, Lim HW, Shin D, Kim H, Kwon B, *et al*. 2012. Enhancement of anti-inflammatory and antinociceptive actions of red ginseng extract by fermentation. *J. Pharm. Pharmacol.* **64**: 756-762.
12. Park BG, Jung HJ, Cho YW, Lim HW, Lim CJ. 2013. Potentiation of antioxidative and anti-inflammatory properties of cultured wild ginseng root extract through probiotic fermentation. *J. Pharm. Pharmacol.* **65**: 457-464.
13. Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**: 248-254.
14. Sherman MP, Aeberhard EE, Wong VZ, Griscavage JM, Ignarro LJ. 1993. Pyrrolidine dithiocarbamate inhibits induction of nitric oxide synthase activity in rat alveolar macrophages. *Biochem. Biophys. Res. Commun.* **191**: 1301-1308.
15. Royall JA, Ischiropoulos H. 1993. Evaluation of 2',7'-dichlorofluorescin and dihydrorhodamine 123 as fluorescent probes for intracellular $H_2O_2$ in cultured endothelial cells. *Arch. Biochem. Biophys.* **302**: 348-355.
16. Jung HJ, Cho YW, Lim HW, Choi H, Ji DJ, Lim CJ. 2013. Anti-inflammatory, antioxidant, anti-angiogenic and skin whitening activities of *Phryma leptostachya* var. *asiatica* Hara extract. *Biomol. Ther.* **21**: 72-78.
17. Elahi MM, Kong YX, Matata BM. 2009. Oxidative stress as a mediator of cardiovascular disease. *Oxid. Med. Cell. Longev.* **2**: 259-269.
18. Gupta SC, Hevia D, Patchva S, Park B, Koh W, Aggarwal BB. 2012. Upsides and downsides of reactive oxygen species for cancer: the roles of reactive oxygen species in tumorigenesis, prevention, and therapy. *Antioxid. Redox Signal.* 16: 1295-1322.
19. Haiyan G, Lijuan H, Shaoyu L, Chen Z, Ashraf MA. 2016. Antimicrobial, antibiofilm and antitumor activities of essential oil of *Agastache rugosa* from Xinjiang, China. *Saudi J. Biol. Sci.* **23**: 524-530.
20. Desta KT, Kim GS, Kim YH, Lee WS, Lee SJ, Jin JS, *et al*. 2016. The polyphenolic profiles and antioxidant effects of *Agastache rugosa* Kuntze (Banga) flower, leaf, stem and root. *Biomed. Chromatogr.* **30**: 225-231.
21. Oh HM, Kang YJ, Lee YS, Park MK, Kim SH, Kim HJ, *et al*. 2006. Protein kinase G-dependent heme oxygenase-1 induction by *Agastache rugosa* leaf extract protects RAW264.7 cells from hydrogen peroxide-induced injury. *J. Ethnopharmacol.* **103**: 229-235.
22. Oh HM, Kang YJ, Kim SH, Lee YS, Park MK, Heo JM, *et al*. 2005. *Agastache rugosa* leaf extract inhibits the iNOS expression in ROS 17/2.8 cells activated with TNF-alpha and IL-1beta. *Arch. Pharm. Res.* **28**: 305-310.
23. Lee HS, Kim MR, Park Y, Park HJ, Chang UJ, Kim SY, *et al*. 2012. Fermenting red ginseng enhances its safety and efficacy as a novel skin care anti-aging ingredient: in vitro and animal study. *J. Med. Food.* **15**: 1015-1023.
24. Chen YS, Liou HC, Chan CF. 2013. Tyrosinase inhibitory effect and

antioxidative activities of fermented and ethanol extracts of *Rhodiola rosea* and *Lonicera japonica*. *The Scientific World Journal* **2013**: 612739.
25. Harini PM, Anegundi RT. 2010. Efficacy of a probiotic and chlorhexidine mouth rinses: a short-term clinical study. *J. Indian Soc. Pedod. Prev. Dent.* **28**: 179-182.
26. Shin YK, Son HU, Kim JM, Heo JC, Lee SH, Kim JG. 2015. *Cinnamomum cassia* bark produced by solid-state fermentation with *Phellinus baumii* has the potential to alleviate atopic dermatitis-related symptoms. *Int. J. Mol. Med.* **35**: 187-194.
27. Miyazaki K, Hanamizu T, Iizuka R, Chiba K. 2003. Bifidobacterium-fermented soy milk extract stimulates hyaluronic acid production in human skin cells and hairless mouse skin. *Skin Pharmacol. Appl. Skin Physiol.* **16**: 108-116.
28. Hsu MF, Chiang BH. 2009. Stimulating effects of *Bacillus subtilis natto*-fermented Radix Astragali on hyaluronic acid production in human skin cells. *J. Ethnopharmacol.* **125**: 474-481.

## 국문초록

**프로바이오틱 유산균 발효에 의한 배초향 잎 추출물의 피부 항산화 및 항염증 활성 증대**

임혜원[1†], 이윤진[2†], 황옥화[1], 윤지영[3], 이수희[4], 김경훈[4*], 임창진[1,5*]
[1]*주)세바바이오텍*
[2]*주)코스모코스*
[3]*전주농생명소재연구원*
[4]*강원대학교 생명과학과*
[5]*강원대학교 생화학과*

본 연구에서, 비발효(ARE) 및 발효(ARE-F) 배초향 잎 열수 추출물의 피부 항산화 및 항염증 효능을 비교함으로써 프로바이오틱 발효의 효과를 검토하였다. ARE-F는 *Lactobacillus rhamnosus* HK-9 발효에 의하여 ARE로부터 제조되었다. In vitro 항염증 효능은 지질다당류(LPS)에 의하여 자극된 HaCaT 각질세포에서 일산화질소(NO), 활성산소종(ROS) 및 유도형 일산화질소 합성효소(iNOS) 분석에 의하여 평가되었다. In vitro antiradical 활성은 2,2-diphenyl-2-picrylhydrazyl radical (DPPH) 소거 측정법에 의하여 평가되었다. LPS 자극에 의하여 증가된 NO, ROS 및 iNOS 수준에 미치는 ARE-F의 감소효과가 ARE에 의한 효과보다 현저히 강하였다. 그러나, ARE와 ARE-F의 DPPH 소거 효능에는 차이가 발견되지 않았다. ARE-F가 증강된 피부 항산화 및 항염증 효능을 갖고 있는 데, 이 결과는 프로바이틱 세균 발효가 배초향 잎의 일부 약리학적 효능을 증가시키는 효과적인 방법일 수 있음을 암시해 주고 있다. 요약하면, 배초향 잎 추출물의 피부 항산화 및 항염증 활성이 프로바이오틱 세균인 *L. rhamnosus* HK-9에 의한 발효에 의하여 증강한다.

http://dx.doi.org/10.4014/mbl.1701.01002