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RESEARCH ARTICLE

Protective Effects of Probiotic-Derived Supernatants Against Oxidative Stress and Inflammation in UVB-Exposed Human Keratinocyte Cells

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Abstract

Probiotics have been reported to reduce reactive oxygen species (ROS) and inflammation, demonstrating antioxidant and anti-inflammatory properties; however, evidence regarding the photoprotective effects of supernatants derived from Thai probiotic strains on human keratinocytes remains limited. This study aimed to evaluate the antioxidant, anti-inflammatory, and UVB-protective effects of probiotic-derived supernatant from eight Thai probiotic strains - *Lactobacillus gasseri* TM01, *L. rhamnosus* TM14, *L. reuteri* TF314, *L. reuteri* TF7, *L. salivarius* MSMC105-3, *L. paracasei* MSMC39-1, *Bifidobacterium animalis* MSMC83, and *B. animalis* TA1 - on UVB-irradiated human keratinocyte (HaCaT) cells. Cells were pretreated with lyophilized probiotic-derived supernatants (0.25 and 0.5 mg/mL) reconstituted in deionized water prior to irradiation with 20 mJ/cm² UVB. Antioxidant activity was assessed by measuring intracellular ROS levels using a fluorometric assay and superoxide dismutase (SOD) activity using a commercial assay kit, while anti-inflammatory effects were determined by quantifying tumor necrosis factor-alpha (TNF-α) levels using ELISA. Pretreatment with probiotic-derived supernatants attenuated UVB-induced photodamage, as evidenced by a reduction in intracellular ROS levels, increased SOD activity (up to 0.288 ± 0.025 U/mL with *L. paracasei* MSMC39-1 at 0.5 mg/mL, vs. 0.056 ± 0.025 U/mL in UVB-control group), and decreased TNF-α levels (as low as 7.000 ± 0.413 pg/mL with *L. paracasei* MSMC39-1, vs. 15.727 ± 1.081 pg/mL in UVB-control group). These findings suggest that Thai probiotic-derived supernatants can mitigate oxidative stress and inflammatory responses in UVB-irradiated keratinocytes, indicating their potential role in photoprotection. In conclusion, these postbiotic preparations demonstrate antioxidant and anti-inflammatory effects that may support the development of UV-protective skincare formulations; however, further mechanistic and *in vivo* studies are required to validate their translational relevance.

Keywords: Probiotics, Keratinocyte, Antioxidant, Anti-inflammatory, Ultraviolet-B-protection

Introduction

Ultraviolet (UV) radiation-induced photodamage to the integumentary system brings about structural changes within the dermal layers, culminating in elevated melanin synthesis, lower skin elasticity, the development of rhytides, and may ultimately be a factor in the emergence of neoplasia [1]. Moreover, UV has been shown to trigger or aggravate various dermatologic conditions, including lupus erythematosus, xeroderma pigmentosum, and chronic actinic dermatitis [2]. Upon exposure of the skin to UVB radiation, there is an activation of enhanced pigment synthesis and incitement of inflammation within epidermal cells or keratinocytes, leading to cellular apoptosis and potentially culminating in the onset of skin cancer [3]. Furthermore, UVB radiation induces the synthesis of reactive oxygen

species (ROS) within cutaneous cells, consequently resulting in oxidative alterations of cellular macromolecules. The oxidative stress stimulated by UVB radiation catalyzes the inflammation of epidermal cells via the mitogen-activated protein kinase (MAPK), nuclear factor erythroid 2-related factor 2 (Nrf-2), and nuclear factor kappa B (NF-κB) signaling pathway, thereby culminating in the production of inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), and interleukin-5 (IL-5) [4]. The employment of antioxidants helps to diminish oxidative stress and decrease the emergence of free radicals elicited by UVB radiation. Many antioxidants, such as vitamin C and polyphenol, are used to combat oxidative injury to the skin. However, they are chemically unstable, easily oxidized, and require high concentrations to achieve efficacy, which may cause irritation. Therefore, more stable biologically active compounds are needed for UV-protection formulations [5].

Probiotics, live microorganisms that confer a health benefit to the host when administered in adequate amounts. The most common probiotics are those in the group of lactic acid bacteria (LAB), which are gram-positive and catalase-negative bacteria. This group includes 2 genera: *Lactobacillus* and *Bifidobacterium* [6]. Research has shown that probiotics possess additional benefits for various disorders, including dermatological allergies and food hypersensitivities [7]. Probiotics have also exhibited potential efficacy in the management of specific dermatological conditions, including psoriasis, atopic dermatitis, and acne [8]. Additionally, it has been found that probiotics also have antioxidant and anti-inflammatory properties. According to Sathikulpakdee *et al.* [9], probiotic-derived lotion showed anti-inflammatory effects in the treatment of mild to moderate acne vulgaris, with efficacy comparable to that of 2.5% benzoyl peroxide. Furthermore, postbiotics, such as probiotic-derived supernatant, are defined as a preparation of inanimate microorganisms and/or their components that confers a health benefit on the host. Unlike probiotics, they offer advantages such as stability across a wide range of temperatures, prolonged shelf life, ease of production, transport, and storage. Postbiotics also minimize safety concerns related to microbial viability [10,11]. Meta-analysis and reviews indicate that topical postbiotics and related microbiome-based therapies demonstrate potential benefits for barrier repair, reduction of inflammation/erythema, and improvement in eczema, acne, and photoaging [9,12,13]. The bioactive components present in probiotic-derived supernatant include short-chain fatty acids, bacteriocins, exopolysaccharides, functional peptides, and organic acids such as lactic acid. These compounds have been reported to exert antioxidant effects by scavenging free radicals and activating endogenous antioxidant pathways (e.g., Nrf2 signaling), as well as anti-inflammatory effects through suppression of NF-κB-mediated cytokine production [14,15]. For example, exopolysaccharides from *Lacticaseibacillus rhamnosus* have been shown to attenuate UV-induced MAPK and NF-κB signaling in HaCaT keratinocytes, reducing UVB-induced ROS production and pro-inflammatory cytokine release [16], providing a mechanistic basis for the photoprotective effects investigated in the current study.

Therefore, probiotics and postbiotics can modulate keratinocyte responses, enhance antioxidant enzyme expression, and improve skin barrier function, supporting their potential as topical photoprotective agents. Based on previously conducted studies, local probiotic strains from local foods and healthy newborns' intestines have shown strong antioxidant and anti-inflammatory properties [9,17-20], but their dermatologic applications, especially their photoprotective activity, have not been investigated. This study aimed to evaluate the antioxidant and anti-inflammatory effects of supernatants derived from Thai probiotic strains on UVB-irradiated human keratinocytes. To our knowledge, this is among the first studies to characterize the photoprotective activity of cell-free supernatants from Thai-origin probiotic strains, including *Bifidobacterium animalis* and *Lactobacillus* spp. not previously investigated in dermatological contexts. These findings may contribute novel, regionally specific data to the growing literature on postbiotic photoprotection and support the development of probiotic-derived active ingredients for UV-protective dermatologic formulations.

Materials and methods

Probiotic strains and probiotic-derived supernatants preparation

Lactobacillus gasseri TM01 (TM1), *Lactobacillus rhamnosus* TM14 (TM14), *Lactobacillus reuteri* TF314 (TF314), *Lactobacillus reuteri* TF7 (TF7), *Lactobacillus salivarius* MSMC105-3 (MSMC105-3), *Lactobacillus paracasei* MSMC39-1 (MSMC39-1), *Bifidobacterium animalis* MSMC83 (MSMC83), and *Bifidobacterium animalis* TA1 (TA1) were obtained from the Center of Excellence in Probiotics, Faculty of Medicine, Srinakharinwirot University, Thailand.

These strains were carefully isolated from Thai local foods and healthy Thai newborns' intestines (see Supplementary). The probiotic strains used in this study were initially cultured in de Man, Rogosa, and Sharpe (MRS) broth and incubated at 37 °C under anaerobic conditions for 48 h. Prior to centrifugation, bacterial growth was confirmed by measuring the optical density at 600 nm (OD_{600}); all cultures were standardized to a cell density of approximately 1×10^9 CFU/mL to ensure consistency across strains before supernatant preparation. Following incubation, the probiotic-derived supernatants were prepared by centrifuging the bacterial cultures at 4,000×g for 15 min at 4 °C. To ensure the complete removal of bacterial cells and debris, the resulting supernatants were carefully collected and passed through a 0.22 μm sterile membrane filter. The bioactive components present in probiotic-derived supernatant of probiotic strains include short-chain fatty acids (propionic acid and butyric acid) and organic acids such as lactic acid (data not shown).

For long-term stability and standardized concentration in subsequent assays, the CFS was subjected to a lyophilization process. The filtered supernatants were first pre-frozen at −80 °C and then transferred to a freeze-dryer (lyophilizer), where they were dried under a high vacuum until a fine powder was obtained. The resulting lyophilized probiotic-derived supernatants were stored in airtight, light-protected containers at 4 °C to preserve the biological activity of the secreted metabolites and bioactive compounds prior to their application on UVB-exposed human keratinocyte cells. Prior to use, lyophilized powders were reconstituted in sterile deionized water to the desired concentration. The pH of the reconstituted supernatants was measured and adjusted to 7.0 - 7.2 using 1 M NaOH or HCl to minimize potential cytotoxic effects attributable to pH rather than bioactive components. To assess potential interference from residual MRS broth components, a MRS broth control (prepared and lyophilized under identical conditions without bacterial inoculation) was included in all cell-based assays.

Keratinocyte cells culture

HaCaT keratinocyte cells were purchased from CLS Cell Line Service GmbH (Eppelheim, Germany). The cells were grown in Dulbecco's modified Eagle medium (DMEM) with 10% heat-inactivated fetal bovine serum and 1% penicillin-streptomycin in a plastic 75 cm^2 flask at 37 °C in a humidified, 5% CO_2 and 95% air atmosphere. The media was changed every 2 days until the cells had grown more than 70% of the flask. The cultures were washed with fresh 0.05% EDTA and were trypsinized with 0.25% (w/v) trypsin-0.53 mM EDTA solution, the cells were used in subsequent experiments at 24 h after seeding.

UVB source and treatment

The UVB source used for simulated solar irradiation was a parallel bank of 50×8-watt tubes from the UVB Bio-Link BLX Cross linker (Bio Sun UVB Vilber Lourmat, France), which emitted UVB radiation in the range of 280 to 320 nm. Before the UVB irradiation, the medium was removed from the cells, and they were washed with phosphate-buffered saline (PBS) at 37 °C. A thin layer of PBS was then applied to the cells. The cells were immediately irradiated with UVB (wavelength 312 nm) from 0 to 50 mJ/cm^2 in a plate without a cover. After UVB exposure, the cells were replenished with fresh medium and incubated further at the specified time points, depending on the parameter being investigated.

Assessment of cell viability

To identify the non-toxic concentration of probiotic-derived supernatant, keratinocyte cells were seeded in 96-well cell culture plates at a density of 1.5×10^4 cells/well. The cells were treated with probiotics-derived supernatant at concentrations ranging from 0.25 to 10 mg/mL and incubated at 37 °C for 24 h.

Following the determination of a non-toxic concentration of probiotic-derived supernatant for the cells, the photoprotective effect of the supernatant was subsequently evaluated. The cells were preincubated for 2 h with concentrations of 0.25 and 0.5 mg/mL of the supernatant, prior to UVB irradiation at 20 mJ/cm^2. After UVB irradiation, probiotic-derived supernatant was removed from 96-well plates and added 0.5mg/mL MTT dissolved with media 100 µL/well and further incubated for 3 h at 37 °C with 5% CO_2. Then, 100% DMSO was added, and absorbance was measured at 570 nm using a microplate reader (BioTek Synergy H1, USA). Cell survival was evaluated as the percentage of cell viability.

Measurement of intracellular ROS

To evaluate intracellular ROS inhibition, 1-step fluorometric intracellular ROS (especially superoxide and hydroxyl radicals) was detected in live cells after irradiated with UVB. Keratinocyte cells were seeded in 96-well cell culture plates at a density of 1.5×10^4 cells/well. The cells were treated with probiotic-derived supernatant in 0.25 and 0.5 mg/mL and incubated at 37 °C for 2 h before irradiation with UVB. Then, fluorometric intracellular ROS kit (Sigma-Aldrich®, USA) was added as mixed solution and further incubated in 5% CO_2, 37 °C incubator for 30 min. Then, fluorescence intensities were measured using a fluorescence microplate reader (BioTek Synergy H1, USA) at an excitation wavelength of 540 nm and an emission wavelength of 570 nm. The result was evaluated as the fold of control.

Measurement of endogenous antioxidant activation

When the cells are in a state of oxidative stress, some antioxidant compounds can increase the levels of endogenous antioxidant enzymes and enhance gene expressions such as SOD can break down the superoxide radical to produce H_2O_2 or O_2. To evaluate the antioxidant enzyme activities, keratinocytes were seeded in 96-well cell culture plates at a density of 1.5×10^4 cells/well. The cells were treated with probiotic-derived supernatant in 0.25 and 0.5 mg/mL and incubated at 37 °C for 2 h before irradiation with UVB. After UVB, the cells were incubated in 5% CO_2, 37 °C incubator for 24 h. Then, keratinocytes and their cell culture medium were collected and analyzed using a superoxide dismutase assay kit (Cayman Chemical, USA). Then, absorbance was measured at 440 nm using a microplate reader (BioTek Synergy H1, USA).

Measurement of anti-inflammatory activity

The keratinocytes were seeded in 96-well cell culture plates at a density of 1.5×10^4 cells/well and treated with probiotic-derived supernatant in 0.25 and 0.5 mg/mL for 2 h under the same experimental conditions as described previously. The cell culture medium was collected and transferred to a precoated ELISA plate; the amount of TNF-α in the cell culture medium was measured by using a human TNF-α ELISA kit (R&D System, USA) according to the manufacturer's protocol. Then, absorbance was measured at 450 nm using a microplate reader (BioTek Synergy H1, USA).

Statistical analysis

Statistical analysis was performed using GraphPad Prism software (version 10.1.0, San Diego, CA, USA). Comparisons between groups were conducted using one-way ANOVA followed by Dunnett's multiple comparison test. All experiments were performed independently in triplicate. Results are presented as mean ± standard deviation (SD). A value of $p < 0.05$ was considered statistically significant.

Results and discussion

Comparison of cell viability

Probiotic-derived supernatants are composed of MRS broth cultured medium; thus, it is imperative to assert that MRS broth does not exert an effect on the cells. The findings indicated that concentrations of 0.25, 0.5, 1, 2, 5, and 10 mg/mL MRS broth cultured medium do not influence the viability of HaCaT cells. Furthermore, the probiotic-derived supernatants (TM1, TM14, TF314, TF7, MSMC105-3, MSMC39-1 MSMC83 and TA1) demonstrated that concentrations of 0.25 and 0.5 mg/mL resulted in cell viability exceeding 80%, indicating the absence of cytotoxicity. Significantly, the concentration that shows the least cytotoxicity is 0.25 mg/mL, as shown in **Figure 1(A)**. For UVB irradiation on the cells, the optimal energy levels for the experiment were determined within the range of 0 - 50 mJ/cm². It was discovered that energy

level of 20 mJ/cm² was the most effective for the experiment, facilitating cell survival rates exceeding 60%, as demonstrated in **Figure 1(B)**, hence the energy of UVB of 20 mJ/cm² was used in the next experiment. After keratinocytes were exposed to 20 mJ/cm² UVB, cell viability was statistically significantly reduced to 66.893 ± 1.486%. In all groups of keratinocytes incubated with probiotic-derived supernatant, there was a statistically significant increase in cell viability compared to UVB-control group, as shown in **Figure 2**.

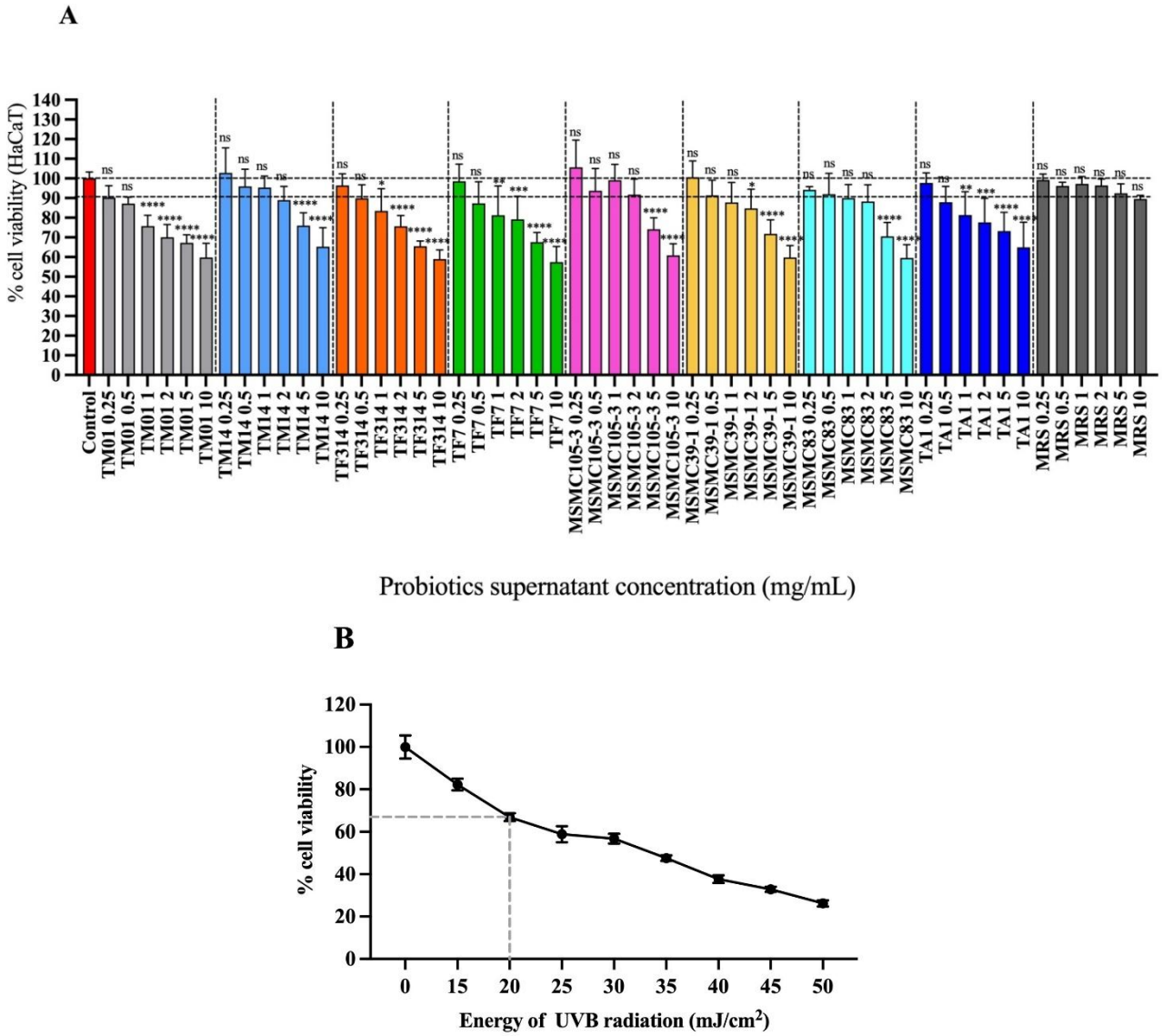


Figure 1 Cytotoxicity of probiotics-derived supernatants on HaCaT cells (A) at various concentrations of each strain of probiotic-derived supernatant and MRS control. (B) at various UVB-energy levels. (A**)** n = 3 /group and **(**B**)** n = 3/energy of UVB radiation. Statistical analysis is by one-way ANOVA with Dunnett's post-test in A. Results are presented as mean ± SD compare with the control group (ns, not statistically significant $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$).

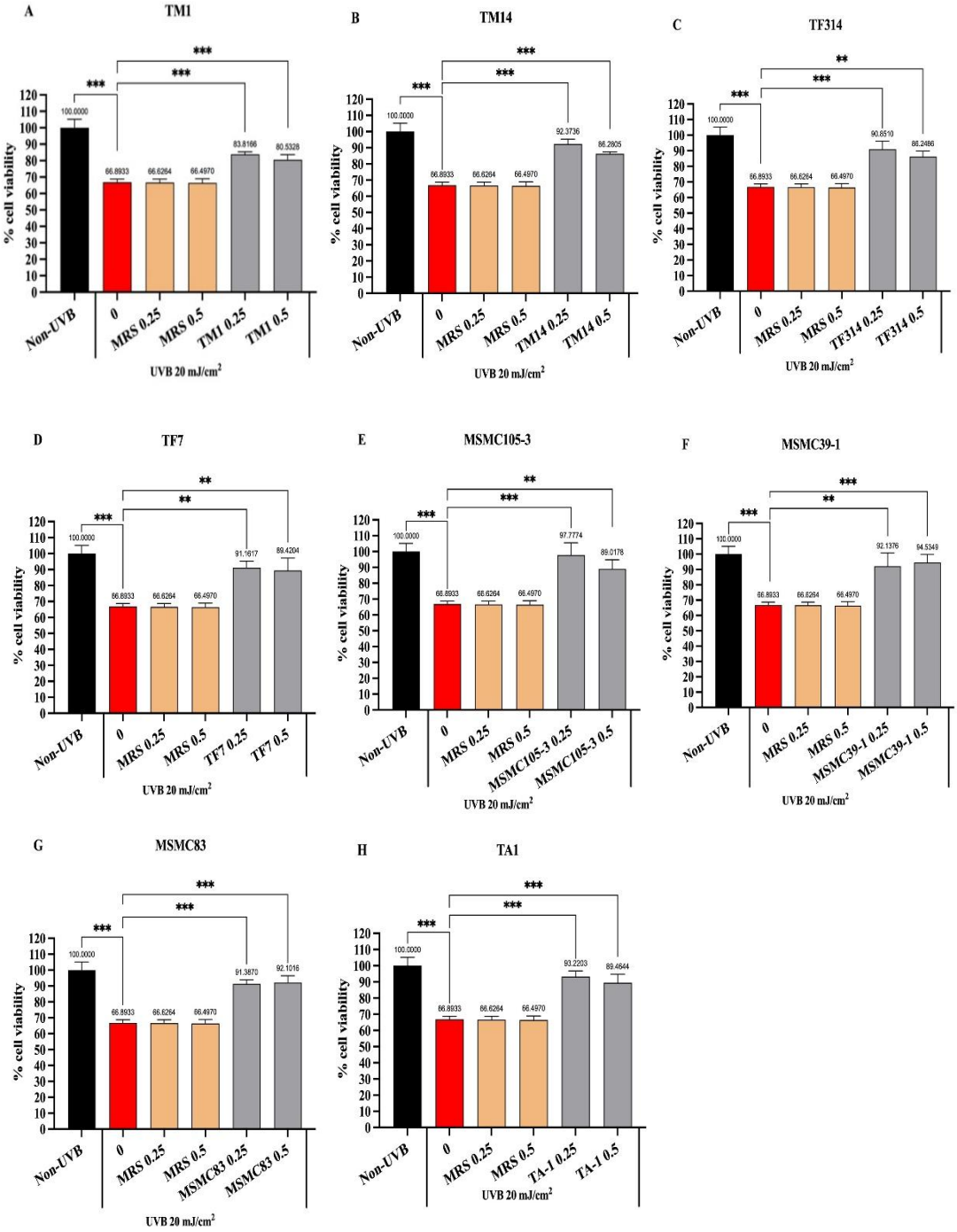


Figure 2 Percentage of cell viability treated with probiotics-derived supernatants (A) TM1, (B) TM14, (C) TF314, (D) TF7, (E) MSMC105-3, (F) MSMC39-1, (G) MSMC83, and (H) TA1 and ultraviolet B on HaCaT cells. (A-H**)** n = 3 /group. Statistical analysis is by one-way ANOVA with Dunnett's post-test in A-H. Results are presented as mean ± SD compare with the UVB control group (**$p < 0.01$, ***$p < 0.001$).

Antioxidant effect of probiotic-derived supernatant on UVB-irradiated human keratinocyte cell line

Since UVB irradiation is known to increase intracellular ROS production and promote oxidative stress in keratinocytes, ROS generation was quantified to evaluate the antioxidant potential of probiotic-derived supernatants. A reduction in ROS levels compared with UVB control group would indicate a protective effect against UVB-induced oxidative damage. Intracellular ROS inhibition was determined by fluorometric intracellular ROS Assay. After the cells were irradiated

with 20 mJ/cm^2 UVB. Intracellular ROS level of UVB-control group of HaCaT cells was 1.000-fold of control. In the group of keratinocytes incubated with 0.25 and 0.5 mg/mL probiotic-derived supernatants, there was a statistically significant decrease in intracellular ROS levels compared to UVB-control group. For 0.25 mg/mL probiotic-derived supernatants, TM14 was the least intracellular ROS level compared to UVB-control group, which was approximately 1.035 ± 0.010 and TA1: 1.041 ± 0.011, TF7: 1.044 ± 0.012, TF314: 1.047 ± 0.013, TM1: 1.049 ± 0.013, MSMC105-3: 1.050 ± 0.008, MSMC39-1: 1.053 ± 0.012, and MSMC83: 1.061 ± 0.009-fold of control, respectively. For 0.5 mg/mL probiotic-derived supernatants, TA1 was the least intracellular ROS level compared to UVB-control group, which was approximately 1.041 ± 0.011 and MSMC83: 1.056 ± 0.009, TF7: 1.056 ± 0.012, TM14: 1.057 ± 0.009, MSMC105-3: 1.057 ± 0.030, MSMC39-1: 1.057 ± 0.012, TF314: 1.060 ± 0.021, and TM1: 1.063 ± 0.013, respectively, as shown in **Figure 3**.

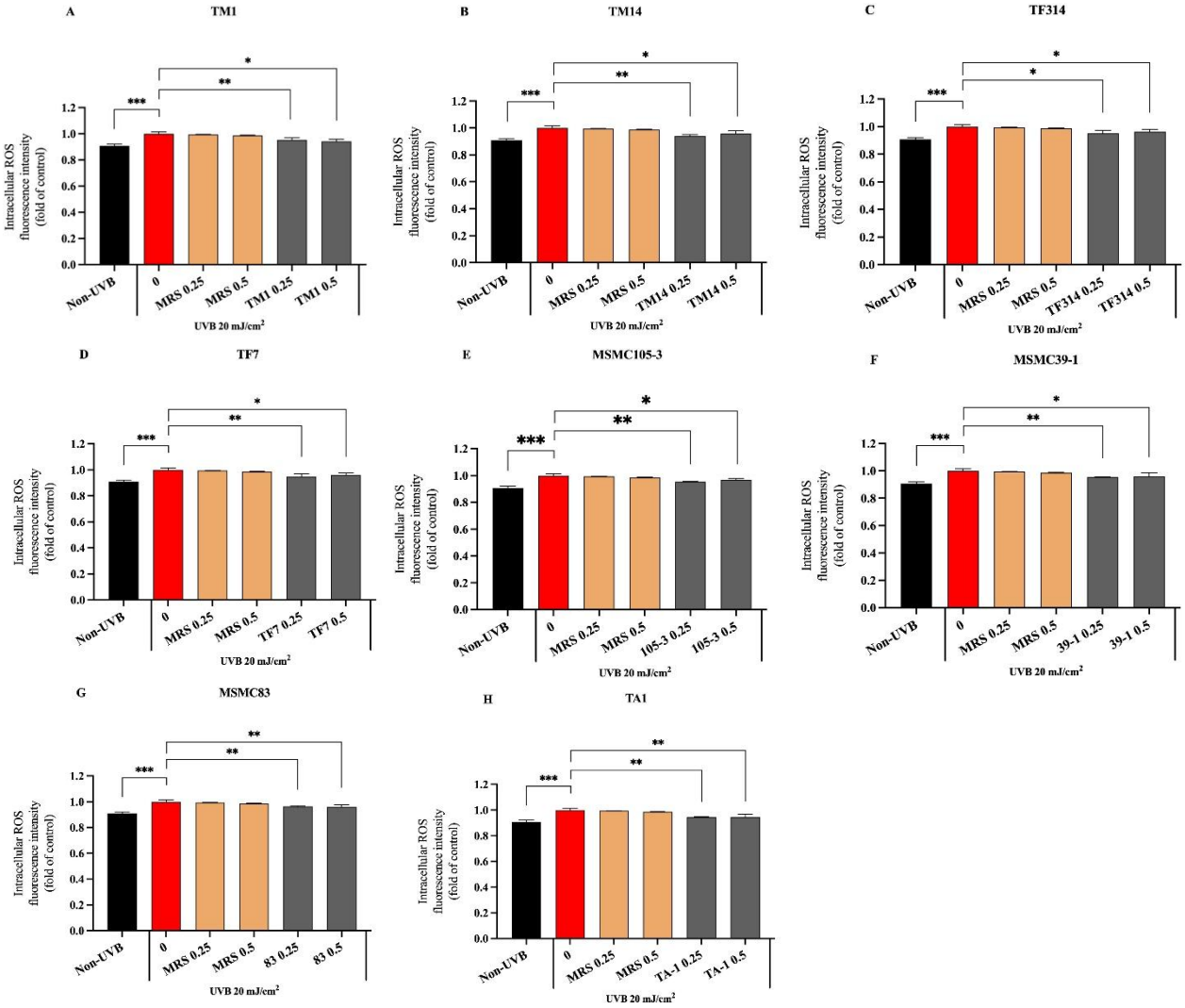


Figure 3 Effect of various probiotic-derived supernatants and UVB on intracellular ROS production in HaCaT cells: (A) TM1, (B) TM14, (C) TF314, (D) TF7, (E) MSMC105-3, (F) MSMC39-1, (G) MSMC83, and (H) TA1. (A-H**)** n = 3 /group. Statistical analysis is by one-way ANOVA with Dunnett's post-test in A-H. Results are presented as mean ± SD compare with the UVB control group (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

For SOD levels, in the group of keratinocytes incubated with 0.25 and 0.5 mg/mL probiotic-derived supernatants, SOD levels were all statistically significantly higher compared to the UVB-control group. SOD level of UVB-control group of HaCat cells was 0.056 ± 0.025 U/mL. For 0.25 mg/mL probiotic-

derived supernatants, TF314 was the highest SOD level compared to UVB-control group, which was approximately 0.249 ± 0.026 U/mL and MSMC39-1: 0.236 ± 0.025, TM14: 0.233 ± 0.026, TA1: 0.232 ± 0.036, MSMC105-3: 0.201 ± 0.026, TF7: 0.194 ± 0.026, MSMC83: 0.190 ± 0.030 and TM1: 0.185 ± 0.027 U/mL, respectively. For 0.5 mg/mL probiotic-derived supernatants, MSMC39-1 was the highest SOD level compared to UVB-control group, which was approximately 0.288 ± 0.025 U/mL and TF314: 0.256 ± 0.026, MSMC105-3: 0.237 ± 0.026, TA1: 0.215 ± 0.036, TF7: 0.184 ± 0.026, MSMC83: 0.176 ± 0.030, TM14: 0.175 ± 0.026 and TM1: 0.168 ± 0.036 U/mL, respectively, as shown in **Figure 4**.

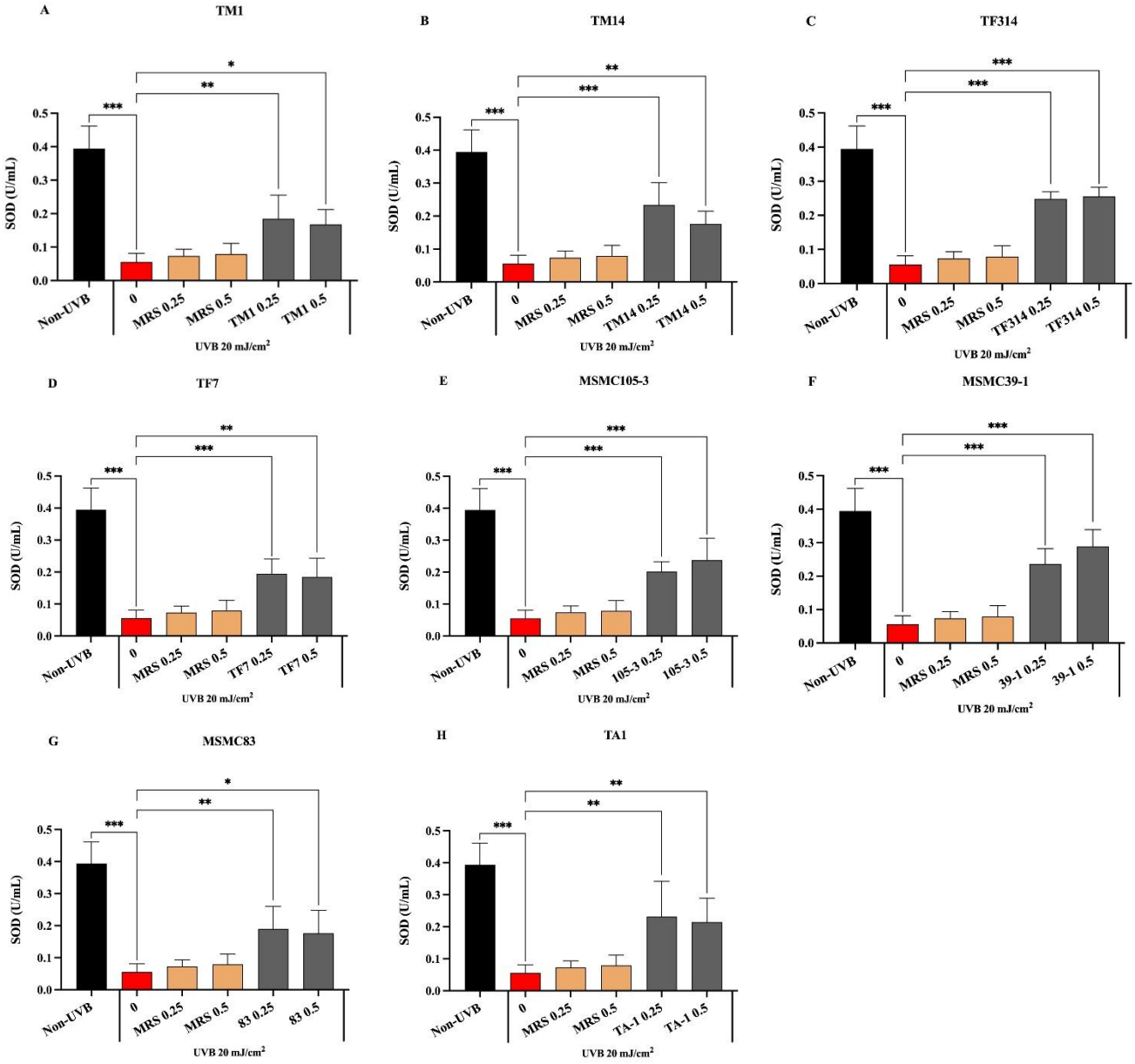


Figure 4 Effect of various probiotic-derived supernatants and ultraviolet B on superoxide dismutase (SOD) level in HaCaT cells: (A) TM1, (B) TM14, (C) TF314, (D) TF7, (E) MSMC105-3, (F) MSMC39-1, (G) MSMC83, and (H) TA1. (A-H) n = 3 /group. Statistical analysis is by one-way ANOVA with Dunnett's post-test in A-H. Results are presented as mean ± SD compare with the UVB control group (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

UVB radiation accelerates skin aging, characterized by fine wrinkles and reduced elasticity. It also stimulates melanogenesis and induces epidermal inflammation, leading to keratinocyte apoptosis and increasing the risk of photocarcinogenesis [21]. UVB irradiation generates intracellular reactive oxygen and

nitrogen species (ROS/RNS) and activates inflammatory pathways, promoting the release of pro-inflammatory cytokines such as TNF-α in keratinocytes [22]. Oral probiotics have demonstrated anti-photoaging effects. Guéniche *et al.* [23] reported that oral administration of *Lactobacillus johnsonii* to subjects exposed to UV radiation prevented the UV-induced reduction in Langerhans cells and promoted recovery of immune homeostasis. Similarly, orally administered probiotics such as *Bifidobacterium breve* and *L. plantarum* have been shown to enhance skin hydration and reduce wrinkle formation by improving skin water content in mice with UV-induced transepidermal water loss [24]. These findings suggest that probiotics may mitigate UV-induced skin damage and photoaging through antioxidant and anti-inflammatory mechanisms.

The present study demonstrates that probiotic-derived supernatants statistically significantly reduce intracellular ROS levels and increase SOD activity in UVB-irradiated keratinocytes, indicating both direct antioxidant effects and activation of endogenous defense pathways. Additionally, all tested supernatants increased cell viability and exhibited anti-inflammatory effects. These findings are consistent with the report by Im *et al.* [25], who showed that *L. acidophilus* IDCC 3302 (ACT 3302) tyndallizate improved cell viability and protected HaCaT keratinocytes from UVB-induced damage. Furthermore, in this study, we investigated cell-free supernatants derived from multiple bacterial strains to compare their protective effects against UVB-induced damage. Regarding cell viability following UVB irradiation, the highest viability was observed with MSMC105-3 at 0.25 mg/mL, followed by TA-1, TM14, and MSMC39-1. In terms of antioxidant activity, intracellular ROS, assessed by a fluorescence-based assay, was most effectively reduced by TM14-derived supernatant at 0.25 mg/mL. In contrast, MSMC39-1-derived supernatant at 0.5 mg/ml produced the greatest increase in SOD levels, indicating activation of endogenous antioxidant defenses.

Probiotics have gained increasing attention for their antioxidant and anti-inflammatory properties. Our group previously reported that *B. animalis* MSMC83, isolated from yogurt and local food products, exhibits significant antioxidant capacity [19]. In a high-fat diet rat model, *B. animalis* MSMC83 upregulated antioxidant enzymes (SOD, GPx, and MDA), increased cholesterol-7α-hydroxylase expression, and reduced liver enzyme levels and proinflammatory cytokines [18].

The mechanisms underlying ROS suppression by probiotic-derived supernatants likely involve multiple bioactive components present in the probiotic-derived supernatant. Lactic acid bacteria are known to secrete antioxidant compounds including exopolysaccharides, short-chain fatty acids, functional amino acids (e.g., glutathione precursors), and antioxidant peptides derived from proteolytic degradation of fermentation substrates [14]. These molecules may scavenge intracellular superoxide and hydroxyl radicals directly, while also activating the Nrf2-Keap1 signaling pathway, which upregulates endogenous antioxidant enzymes including SOD, catalase, and glutathione peroxidase [26]. Notably, the probiotic-derived supernatant of skin-derived bacteria has been demonstrated to activate Nrf2 and HO-1 protein expression in primary human keratinocytes, thereby reducing intracellular ROS levels [27]. The observed increase in SOD activity in the present study supports activation of this endogenous defense pathway. Furthermore, exopolysaccharides from lactic acid bacteria have been shown to directly attenuate UVB-induced ROS overproduction and suppress MAPK phosphorylation cascades in HaCaT cells [16,28], consistent with the antioxidant and anti-inflammatory findings of the current study.

Anti-inflammatory effect of probiotic-derived supernatant on UVB-irradiated human keratinocyte cell line

Because UVB irradiation activates MAPK and NF-κB signaling pathways, leading to increased expression of pro-inflammatory cytokines, TNF-α levels were measured to assess the degree of UVB-induced inflammation and the potential anti-inflammatory effects of probiotic-derived supernatants. After the cells were irradiated with 20 mJ/cm^2 UVB. The TNF-α level of UVB-control group of HaCaT cells was 15.727 ± 1.081 pg/mL. In the group of keratinocytes incubated with probiotic-derived supernatants, there was a statistically significant reduction in TNF-α levels compared to UVB-control group. For 0.25 mg/mL probiotic-derived supernatants, TA1 was the least TNF-α level compared to UVB-control group, which was approximately 7.771 ± 1.102 pg/mL and MSMC83:

9.386 ± 1.443, MSMC105-3: 9.432 ± 2.908, TM1: 10.079 ± 0.854, MSMC39-1: 10.292 ± 1.006, TF7: 10.967 ± 1.807, TM14: 11.265 ± 1.974, and TF314: 11.491 ± 1.527 pg/mL, respectively. For 0.5 mg/ml probiotic-derived supernatants, MSMC39-1 was the least TNF-α level compared to UVB-control group, which was approximately 7.000 ± 0.413 pg/mL and MSMC105-3: 7.650 ± 0.968, TA1: 7.791 ± 0.329, MSMC83: 7.975 ± 1.679, TM14: 8.642 ± 0.691, TF7: 8.885 ± 0.753, TF314: 9.195 ± 0.581, and TM1: 9.530 ± 1.733 pg/mL, respectively, as shown in **Figure 5**.

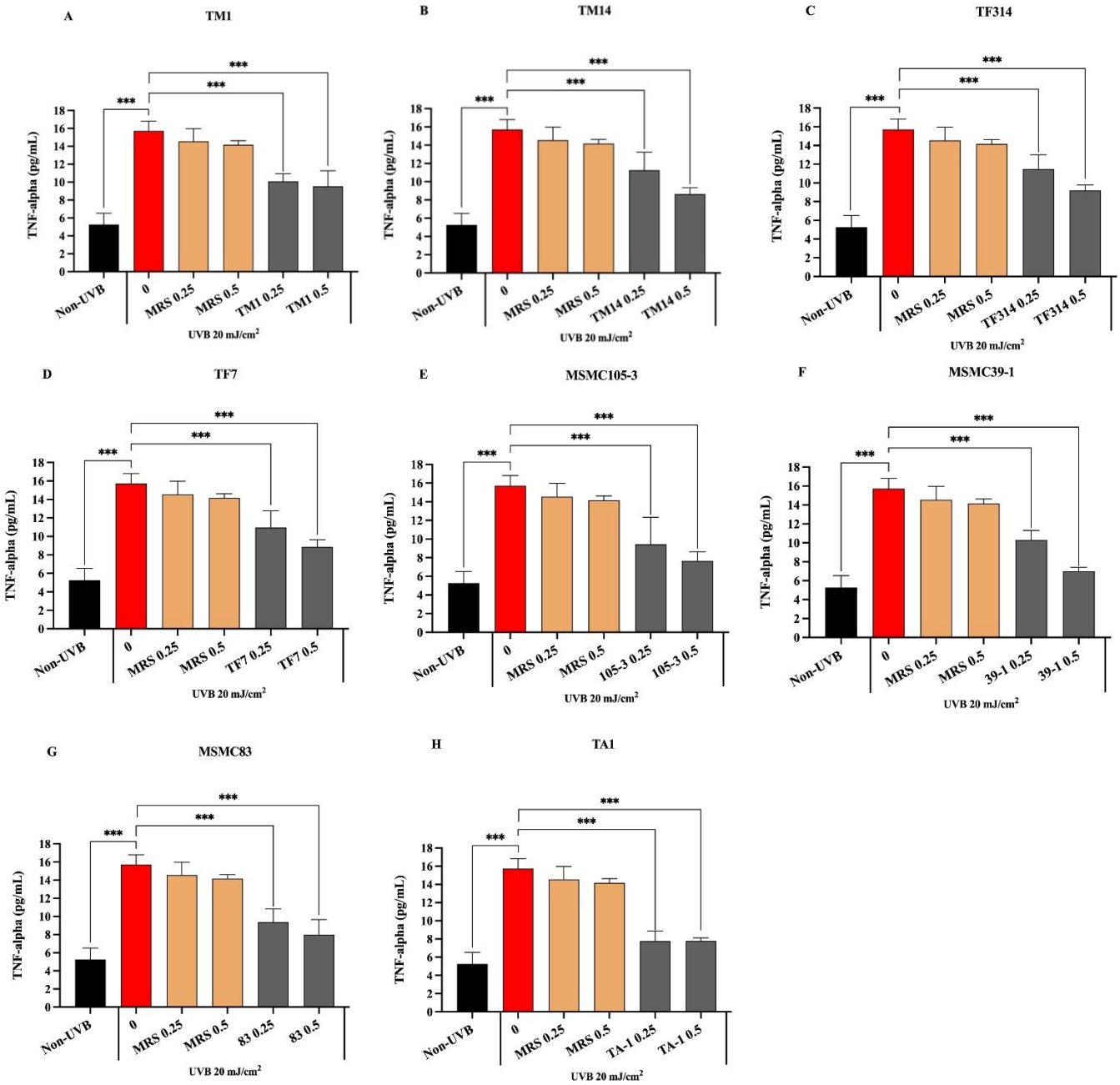


Figure 5 Effect of various probiotic-derived supernatants and ultraviolet B on TNF-α level in HaCaT cells: (A) TM1, (B) TM14, (C) TF314, (D) TF7, (E) MSMC105-3, (F) MSMC39-1, (G) MSMC83, and (H) TA1. (A-H) n = 3/group. Statistical analysis is by one-way ANOVA with Dunnett's post-test in A-H. Results are presented as mean ± SD compare with the UVB control group (***$p < 0.001$).

The present study demonstrates that probiotic-derived supernatants statistically significantly reduce inflammatory effects in UVB-irradiated keratinocytes. MSMC39-1-derived supernatant at 0.5 mg/mL

demonstrated the strongest inhibitory effect on TNF-α production. Overall, our findings suggest that the MSMC39-1-derived supernatant appears to be the most suitable candidate for UVB protection among those evaluated. Regarding our group previously reported that wound irrigation with probiotic-derived supernatant of *L. paracasei* MSMC39-1 following impacted mandibular third molar removal significantly reduced TNF-α levels in gingival crevicular fluid compared with controls.

In the present study, we focused on bacterial metabolites (postbiotics) rather than live bacteria, as they offer greater stability and reduced safety concerns related to microbial viability [10,11]. Prior studies have shown that probiotic metabolites, such as exopolysaccharides, lactic acid, and short-chain fatty acids, exert antioxidant and anti-inflammatory effects [14,15]. In particular, exopolysaccharides can activate Nrf2-mediated antioxidant responses and suppress NF-κB–dependent cytokine production [29,30]. The reduction in TNF-α observed in our study further supports the anti-inflammatory potential of these postbiotic preparations.

The present study provides preliminary *in vitro* evidence that probiotic-derived supernatants can attenuate UVB-induced oxidative stress and inflammatory responses in HaCaT keratinocytes. These findings should be interpreted within the constraints of the experimental model and do not, at this stage, support broader claims regarding clinical or cosmeceutical utility. Whether these *in vitro* effects translate to meaningful photoprotection in living skin remains to be established through further mechanistic, *in vivo*, and clinical studies.

Limitations

This *in vitro* study using HaCaT keratinocytes may not fully reflect human skin responses. The limited biomarker assessment highlights the need for further mechanistic and *in vivo* studies to establish translational relevance. Additional limitations include the exclusive use of a single immortalized cell line and the absence of mechanistic pathway analyses (e.g., Nrf2/NF-κB signaling). Future studies should incorporate primary keratinocytes or skin models and pathway-specific assays to further validate the observed effects.

Conclusions

Probiotic-derived supernatants demonstrated antioxidant, anti-inflammatory, and cytoprotective effects against UVB-induced damage in keratinocytes *in vitro*. The MSMC39-1-derived supernatant showed the most effective protective profile. These findings highlight postbiotics as promising candidates for photoprotective and anti-photoaging applications.

Ethics statement

Ethical approval was not required for this study because the experiments were conducted using commercially available human keratinocyte cell lines (HaCaT) and did not involve human participants or animal subjects.

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ChatGPT was used to assist in improving the wording and phrasing of this manuscript. All scientific content, analyses, and conclusions were independently developed by the authors.

CRediT author statement

Wichayut Pholprasitthito: Conceptualization, Methodology, Validation, Writing - Original Draft. **Salinee Rojhirunsakool**: Formal Analysis, Data Curation, Writing - Review & Editing. **Chantanapa Chantarangkul**: Methodology, Validation, Investigation, Resources, Visualization. **Paiwan Buachan**: Methodology, Validation, Investigation, Resources. **Montree Udompataikul**: Writing - Review & Editing, Visualization. **Malai Taweechotipatr**: Conceptualization, Methodology, Supervision, Project administration, Funding acquisition, Writing - Review & Editing.

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Supplementary Materials

Probiotics strains

Lactobacillus gasseri TM01 (TM1) and *Lactobacillus rhamnosus* TM14 (TM14) were carefully isolated from raw milk samples, *Lactobacillus reuteri* TF314 (TF314) was carefully isolated from fermented chicken sausages, *Lactobacillus reuteri* TF7 (TF7) was carefully isolated from pickled olives, *Lactobacillus salivarius* MSMC105-3 (MSMC105-3), *Lactobacillus paracasei* MSMC39-1 (MSMC39-1), and *Bifidobacterium animalis* MSMC83 (MSMC83) were carefully isolated from healthy Thai newborns' intestines and *Bifidobacterium animalis* TA1 (TA1) was carefully isolated from raw cow milk samples.