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"The fermented milk by *Streptococcus thermophilus* CGMCC 24468 prevents oxidative damage of H_2O_2 to HaCaT via modulating MAPK/ERK signaling pathway"

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1. Introduction

S. thermophilus, a thermophilic lactic acid bacterium, is recognized as the second most significant industrial starter culture after Lactococcus lactis[1]. Beyond its traditional use in dairy fermentation, research indicates that *S. thermophilus* possesses several biological properties essential for survival in the gastrointestinal tract (GIT), including bile salt and acid tolerance, as well as the ability to adhere to host intestinal tissues[2-3]. These findings highlight its potential as a promising probiotic candidate [4].

The skin, being the body's outermost layer, is susceptible to various environmental factors, including temperature fluctuations, pollution, and ultraviolet (UV) radiation. Among these, UV radiation is recognized as the primary extrinsic factor contributing to skin aging, commonly designated as photoaging[5], accounting for up to 80% of facial aging [6]. UV radiation directly damages DNA and stimulates the production of reactive oxygen species (ROS), leading to lipid peroxidation, degradation of extracellular matrix components, and inflammation[7]. To combat oxidative stress, organisms have evolved various defense mechanisms involving key antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) [8].

Probiotics, defined as live microorganisms, are excellent antioxidative agents providing health benefits to the host. Probiotics and their bioactive components (postbiotics), including bacterial lysates and fermented filtrates, have gained attention in skincare formulations due to their abilities to modulate skin microecology, decelerate skin aging, and prevent photoaging through the mechanisms such as generating antioxidative molecules, scavenging free radicals, reducing matrix metalloproteinase (MMP) expression, and inhibiting inflammatory cytokines [9]. Currently, numerous cosmetic products are being commercialized with claims of containing probiotics or postbiotics[10]. Notably, one well-known probiotic is "Bifida ferment lysate," an ultrasound-inactivated suspension derived from *Bifidobacterium longum*, which has demonstrated efficacy in enhancing skin barrier function while alleviating cutaneous sensitivity [11].

Compared to other lactic acid bacteria, *S. thermophilus* is highly adapted to grow in milk[12]. *S. thermophilus* produces bioactive metabolites such as gamma-aminobutyric acid (GABA)[13], folate[14], glutathione[15], bioactive peptides and free amino acids[16]. Additionally, *S. thermophilus* also produces bacteriocins[17], exopolysaccharides (EPS) including hyaluronic acid, and SOD during its growth[18]. Consequently, the fermented milk produced by *S. thermophilus* serves as a multifunctional ingredient that provides nutrients for the skin and its microbiome, while also functioning as a preservative and antioxidant in cosmetic emulsions.

In this study, a strain of *S. thermophilus* CGMCC24468, previously isolated from traditional Chinese fermented milk, exhibits strong free radical scavenging abilities. To assess the beneficial effects of the milk fermented by strain CGMCC24468 on skin aging, HaCaT cells were utilized as a model to determine the expression of biomarker genes related to skin barrier function and transcriptional levels of antioxidative enzyme genes when co-cultivated with the fermented milk. The protective effects of the fermented milk under conditions of H_2O_2 exposure were evaluated by measuring intracellular ROS levels and the inhibitory effect on the mitogen-activated protein kinases (MAPK) signaling pathway. The aim of this study is to elucidate the protective mechanism of the fermented milk against ROS-induced damage, thereby providing evidence for its potential use as a natural ingredient in cosmetics to combat photoaging.

2. Materials and Methods

2.1 Strains, Cultivation Conditions, and Sample Preparation

S. thermophilus strains used in this study were originally isolated from traditional yogurt sourced from Inner Mongolia, China, and deposited in the China General Microbiological Culture Collection Center (CGMCC) under strain numbers CGMCC 7.179, CGMCC 24468, and CGMCC 25263, respectively. These strains were routinely propagated in M17 broth supplemented with 1% (wt/vol) lactose (LM17 broth) at 42°C under static conditions. The Bifidobacteria animalis CGMCC25262 was isolated from the feces of healthy adults and routinely cultured in de Man, Rogosa and Sharpe (MRS) broth with 0.05% cysteine, statically at 37°C for 36 h[19].

After overnight incubation, each strain was inoculated into 5 mL of fresh LM17 broth or MRS at a 2% inoculum volume and incubated statically at 42°C or 37°C for 12 h. Filtered through a sterile 0.22 μm filter and obtained the cell-free fermented filtrate (CFFF, labeled as sample 1). The bacterial cells were harvested, resuspended in 5 mL of PBS and divided into two parts, one was labeled as sample 2, the other was subjected to ultrasonic disruption (450 W, 5-second pulses on ice with 5-second intervals) until no colony formation was observed on LM17 agar plates. Following disruption, the suspensions were centrifuged at 10,000 rpm for 10 min to remove cell debris, then filtered through a sterile 0.22 μm filter, reference to as sample 3. All samples were used for DPPH scavenging assays.

To prepare the sonicated fermented milk lysate (SFML), 2% overnight cultures of *S. thermophilus* were inoculated into 50 mL of sterilized milk (8% reconstituted milk powder containing 2% sucrose, sterilized at 105°C for 10 min) and incubated at 42°C for 12 h, followed by storage at 4°C for 12 h. Subsequently, the fermented milk was ultrasonicated (450 W, 5-second pulses on ice with 5-second intervals) until no colony formation was observed on LM17 agar plates. The samples were then centrifuged at 10,000 rpm for 10 min, and the supernatant was collected and filtered through a 0.22 μm filter, and then stored at 4°C.

The human keratinocyte line HaCaT was cultured in High-Glu DMEM complete medium supplemented with 10% (vol/vol) fetal bovine serum and 1% (vol/vol) penicillin/streptomycin solution (100 U/mL penicillin and 100 μg/mL streptomycin) at 37°C in a humidified atmosphere containing 5% CO_2. Cell experiments were initiated when the cell confluence reached approximately 80%.

2.2 Scavenging Abilities of S. thermophilus Strains on DPPH Radicals

The scavenging activities of *S. thermophilus strains* on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals were analyzed according to a previously described method with minor modifications[20]. The DPPH radical scavenging activity was calculated using the formula: $(1 - A_{sample}/A_{control}) \times 100\%$.

2.3 Cell Viability Assay

HaCaT cells were seeded in 96-well plates at a concentration of 5×10^3 cells/well and incubated for 24 h at 37°C. Various concentrations of SFML were added to a plate. Meanwhile, serum-free DMEM diluted H_2O_2 solutions at different concentrations were added to 96-well

plates.After 24 h of incubation,the optical density (OD) at 450 nm was measured to assess cell viability according to the instructions of CCK-8. Cell viability was calculated as follows: Relative cell viability = (A_{sample} / $A_{control}$)×100%.

2.4 Intracellular ROS Detection in HaCaT Cells

HaCaT cells were seeded in 6-well plates at a density of 2×10^5 cells/well. 2 mL of 0.6%, 1.2%, and 2.5% SFML diluted with serum-free DMEM medium were added to the wells and co-incubated for 24 h, respectively. Then the cells were collected via centrifugation at 6000 rpm for 5 min, washed three times with PBS, and subsequently treated with 2 mL of 0.75 mM H_2O_2 in for 3 h. After removing the H_2O_2 solution, 10 μM 2',7'-Dichlorodihydrofluorescein diacetate (DCFH-DA) was added to the cells and incubated at 37°C for 20 min. The cells were then washed with PBS buffer, and intracellular ROS levels were observed using fluorescence microscopy. Untreated HaCaT and those treated solely with 0.75 mM H_2O_2 served as control.

2.5 RNA extraction and quantitative Real-time PCR

HaCaT cells were seeded in 6-well plates at a density of 2×10^5 cells/well and incubated for 24 h. After washing with PBS, 2 mL of 0.6%, 1.2%, and 2.5% SFML diluted with serum-free DMEM medium were added to the wells and co-incubated for 24 h, respectively. In the H_2O_2-induced damage model, 0.75 mM H_2O_2 diluted with serum-free DMEM was added to the HaCaH cells pre-cultivated with SFML mention above and incubated for 3 h. Total RNA from the HaCaT cells was extracted using the SPAPKeasy Cell RNA Kit, and quantitative real-time PCR (qPCR) was performed using 2×Universal SYBR Green Fast qPCR Mix, β-actin was used as a reference gene, and relative gene transcription levels were calculated using the 2-ΔΔCT method. Primers are listed in Table 2.

Table 2. primer sets used for qPCR

Gene	Forward (5'-3')	Reverse (5'-3')
β-actin	TACATCACTATTGGCAACGAGC	GTCGGATGTCAACGTCACACTT
CAT	AGTGATCGGGGGATTCCAGA	GAGGGGTACTTTCCTGTGGC
SOD	AAAGATGGTGTGGCCGATGT	CAAGCCAAACGACTTCCAGC
GPX	AGTCGGTGTATGCCTTCTCG	TCTTGGCGTTCTCCTGATGC
MAPK-1	CAGTTCTTGACCCCTGGTCC	GTACATACTGCCGCAGGTCA
AP-1	GCTGCTCTGGGAAGTGAGTT	TTTCTCTAAGAGCGCACGCA
MMP-1	CACAGCTTTCCTCCACTGCT	CCGCTTTTCAACTTGCCTCC

2.6 Western Blot

The HaCaT cells were cultured according to the method described in 2.5. The cell lysates were prepared using Western and IP lysis buffer supplemented with phosphatase inhibitors (MCE) and phenylmethylsulfonyl fluoride (PMSF). Cells were lysed on ice for 10 min, then centrifuged at 13,000×rpm for 10 min at 4°C, and the supernatant was collected as the total protein. Proteins were separated by SDS-PAGE, transferred to a PVDF membrane, and blocked with 5% skim milk at room temperature for 1h. The membrane was incubated overnight at 4°C with primary antibodies, followed by horseradish peroxidase-conjugated secondary antibodies for 1h at room temperature. Images were captured using the Amersham Imager 680, and the results were processed and analyzed using ImageJ software.
The antibodies F11R (JAM-1) , Occludin (OCLN), Claudin (CLDN-1) were purchased from ABclonal. The antibodies ERK1/2, Phosphorylated-ERK1 (T202/Y204) + ERK2T185/Y187 were obtained from Abmart . The antibodies β-actin and the antibody HRP Goat Anti-Rabbit IgG were acquired from ABclonal.

2.7 Scratch wound healing assay

HaCaT cells were seeded in 6-well plates at a density of 5×10^5 cells/well and incubated until the confluence reached approximately 90-100%. The culture was then replaced with 1 mL of PBS. Using a 200 μL pipette tip, vertical scratches were made along the bottom of each well. The cells were subsequently treated with 5% SFML and incubated for 24 h. For the control

groups, 2 mL of serum-free DMEM alone served as the normal control, while 2 mL of serum-free medium containing epidermal growth factor (EGF, 1 μg/mL) was used for the positive control. Images of cell migration were captured using an inverted microscope at two time points: immediately after scratch creation and 24 h post-treatment.

2.8 Statistical analysis

Data are presented as means ± standard deviation (SD) of three independent experiments (n = 3). Statistical differences were determined using one-way ANOVA followed by Dunnett's multiple comparison tests. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. The Antioxidant Ability of S. thermophilus CGMCC24468 in vitro

S.thermophilus is a facultative anaerobic bacterium that *S.thermophilus* has evolved efficient inducible defense systems against oxidative stress. Superoxide dismutase is widely distributed among aerobic microorganisms to convert superoxide anions into molecular oxygen and hydrogen peroxide. It has been reported that *S.thermophilus* contains a single manganese-containing superoxide dismutase (MnSOD), and the expression of SodA gene is not regulated by O2. In this study, we identified the presence of the SodA gene in the genomes of *S.thermophilus* strains CGMCC 7.179, CGMCC 25263, and CGMCC 24468, with the whole DNA sequence 600 bp in size. The deduced amino acid sequences of MnSODs from these three strains were 100% identical, indicating that these strains possess potential MnSOD activity. Multiple sequence alignments revealed that MnSOD from strain CGMCC 24468 shared 97.01%, 85.07%, and 50.48% identity with *S.salivarius* JIM 8777, *S.oralis ATCC 35037*, and *E. coli MG 1655*, respectively (Figure 1).

2,2-diphenyl-1-picrylhydrazyl (DPPH) is a stable free-radical molecule commonly used in antioxidant assays. In this study, we evaluated the DPPH scavenging activity of bacterial cells, bacterial lysates, and cell-free fermented filtrate (CFFF) derived from *S.thermophilus* strains CGMCC 7.179, CGMCC 25263, and CGMCC 24468. Additionally, a SOD-deficient B. animalis strain CGMCC 25262 served as a control.

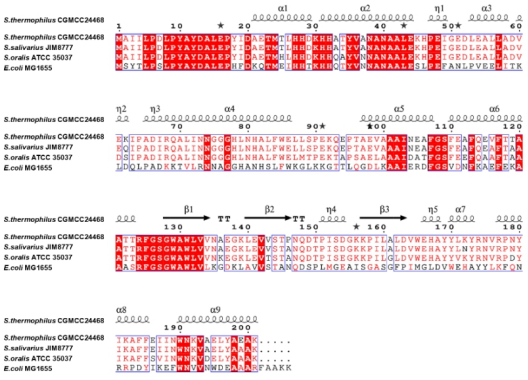


Figure 1. Multiple-sequence alignment of superoxide dismutases from strain CGMCC 24468 and other bacteria. All sequences are displayed as full-length sequences before processing. The secondary structure of SOD is shown at the top of each set of sequences. Abbreviations: α, α-helix; β, β-sheet; η, η-helix;. Perfectly matched residues are displayed in white on red. Similar amino acid residues are boxed with a thin line.

As shown in Table 1, all tested samples exhibited scavenging activity against DPPH, with CFFF demonstrating significantly higher scavenging ability compared to the other bacterial samples. Notably, bacterial cell samples showed stronger scavenging activity than bacterial lysate, possibly due to the destruction or removal of bioactive molecules during sonication or filtration processes. Importantly, the majority samples of *S.thermophilus* exhibits higher scavenging activities than that of *B.animalis* CGMCC 25262, suggesting that antioxidative enzymes involved in *S.thermophilus* play a critical role in eliminating free radicals in vitro.

Table 1. Scavenging free racial abilities of the *S.thermophilus* samples against DPPH

	Scavenging rate of DPPH(%)			
sample	CGMCC 25262	CGMCC 7.179	SDMCC 050254	CGMCC 24468
Bacterial cells	6.85±1.78	9.67±0.74	7.24±1.25	10.65±2.39
Bactrial Lysates	3.25±1.34	4.94±1.56	5.99±1.20	4.97±2.22
CFFF	56.88±0.89	56.84±1.19	63.62±0.93	73.79±0.50

All samples were diluted 10 times before used. CFFF, cell-free fermented filtrates

3.2 Cell Viability of HaCaT Cells

During milk fermentation, *S.thermophilus* produces lactic acid as the primary end-product, leading to a decrease in pH value. Additionally, it also generates metabolites such as acetaldehyde, diacetyl, and bacteriocin, contributing to the aroma and shelf life of dairy fermented products. To evaluate the potential toxicity of these metabolites on HaCaT, the viability of HaCaT cells was assessed after co-cultivation with various concentrations (2.5%, 1.2%, 0.6%, 0.3%, 0.15%, 0.07%, 0.03%) of the SFML produced by strain CGMCC 24468.

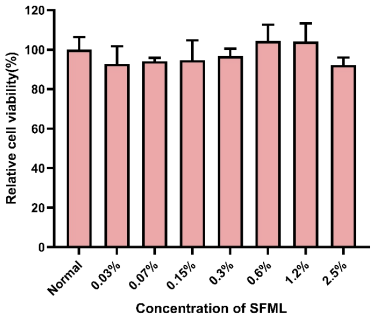
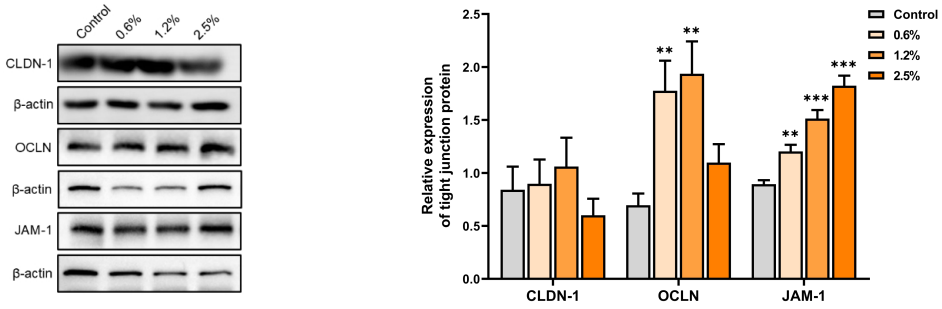


Figure 1. The relative survival of HaCaT cells with various treatments of SFML by *S.thermophilus* CGMCC24468. The survival of the HaCaT cells without treatment was defined as 100%. Each group is provided with six multiple holes.

3.3 Enhancement of Tight Junction Protein Expression levels by SFML

Skin barrier dysfunction plays a crucial physiological role in the development of skin diseases. Tight junction (TJs) proteins are multifunctional elements involved in the barrier function of the skin, regulation of epidermal homeostasis, and differentiation of the epidermis. When HaCaT cells were co-incubated with 0.6%, 1.2%, and 2.5% SFML, the expression levels of tight junction proteins (CLDN-1, OCLN, and JAM-1) were increased compared to untreated HaCaT cells. Particularly, 0.6% and 1.2% SFML increased the expression levels of OCLN by 2.55-fold and 2.78-fold, and JAM-1 by 1.34-fold and 1.69-fold, respectively ($p < 0.05$), indicating the protective effects of the fermented milk on HaCaT cells (Figure 3).



(**a**) (**b**)

Figure 3. SFML enhanced the expression levels of the tight junction protein genes. (a) The protein content of the CLDN-1, OCLN, JAM-1 and β-actin in HaCaT cells after co-incubated with various contents of SFML by Western blot. Each line represents different treatments. (b) Semiquantitative analysis of the relative expression levels of tight junction protein in corresponding bands. The control group was treated only with serum-free DMEM medium. Grayscale values are processed by Image J. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

3.4 Construction of an Oxidative Stress Damage Model in HaCaT Cells

To construct an oxidative stress damage model, HaCaT cells were exposed to varying concentrations of H_2O_2 (0.05 mM, 0.1 mM, 0.25 mM, 0.5 mM, 0.75 mM, 1 mM, 2.5 mM, 5 mM, and 10 mM) for 3 hours, and cell viability was determined under those conditions. As shown in Figure 4a, cell viability decreased with H_2O_2 concentration increasing, 0.75 mM H_2O_2 exhibited a significant inhibitory effect on HaCaT cell proliferation. Therefore, 0.75 mM H H_2O_2 was selected for subsequent experimental analysis.

To evaluate the cytoprotective effects of the fermented milk produced by *S.thermophilus* CGMCC 24468 against H_2O_2 -induced oxidative stress, co-cultivated HaCaT cells were challenged to 0.75 mM H_2O_2 after treatment with various concentrations of SFML (2.5%, 1.2%, 0.6%, 0.3%, 0.15%, 0.07%, and 0.03%). The results showed that cell viability increased with increasing SFML concentration, particularly at 0.6% and 1.2% SFML, where cell proliferation rate was restored to levels comparable to those without H_2O_2 exposure, suggesting the fermented milk has the potential to mitigate the damage induced by H_2O_2 (Figure 4b).

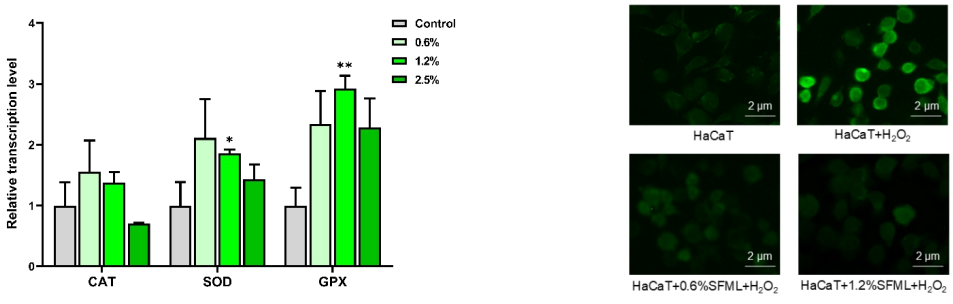
(**a**) (**b**)

Figure 4. HaCaT cell viabilities with various treatments. (a) The viabilities of the HaCaT cells with various contents of H_2O_2; (b) The viabilities of the precultivated HaCaT cells with various contents of SFML before exposure to H_2O_2. Normal, HaCaT cells without any treatment; Control, HaCaT cells treated with H_2O_2 alone.

3.5 Fermented Milk Enhanced the Transcription Levels of Antioxidative Enzymes

When co-culture with various content SFML, all tested samples significantly increased the transcription levels of antioxidative enzyme genes in HaCaT cells. Specifically, concentrations of 0.6% and 1.2% SFML upregulated the Catalase (CAT) gene by 1.55-fold and 1.37-fold, the SOD gene by 2.11-fold and 1.85-fold, as well as the glutathione peroxidase (GPX) gene by 2.34-fold and 2.92-fold, respectively (Figure 5a).

Subsequently, HaCaT cells pretreated with SFML were exposed to 0.75 mM H_2O_2, and intracellular ROS levels were assessed using fluorescence microscopy (Figure 5B). The fluorescence intensity in HaCaT cells treated with H_2O_2 alone was markedly higher than that of control group. Pre-incubation with 0.6% and 1.2% SFML prior to H_2O_2 exposure notably reduced the fluorescence intensity to levels comparable to the control group, suggesting that the fermented milk enhanced the ability of HaCaT cells to eliminate ROS. This finding is consistent with the observed upregulation of antioxidative enzyme gene transcription levels induced by the SFML.



(**a**) (**b**)

Figure 5. SFML activated the antioxidative abilities against H_2O_2 in HaCaT cells. (a) SFML enhanced the transcription levels of antioxidative enzyme genes; (b) ROS levels in HaCaT cells observed using a fluorescence microscope.

3.6 Fermented Milk Inhibited the MAPK Signaling Pathway

Mitogen-activated protein kinases (MAPKs) constitute a family of evolutionarily conserved serine/threonine protein kinases. Extracellular signal-regulated kinase (ERK), also known as MAPK-1, is a member of the MAPK family that mediates signal transduction pathways involved in cell growth, differentiation, adaptation to environmental stress, and other critical cellular physiological and pathological processes. To investigate the protective mechanism of fermented milk against oxidative stress, HaCaT cells were pre-incubated with SFML at concentrations of 0.6% and 1.25% before exposure to H_2O_2. The results demonstrated that exposure to H_2O_2 alone significantly increased the transcription level of MAPK-1 gene, leading to the activation of downstream target genes such as activator protein-1 (AP-1) and matrix metalloproteinase-1 (MMP-1). This indicated that H_2O_2 activated the MAPK signaling pathway. However, when HaCaT cells were pre-incubated with SFML prior to H_2O_2 exposure, the increase in AP-1 and MMP-1 gene transcription levels induced by H_2O_2 was prevented due to the inhibition of MAPK signaling, particularly at a concentration of 0.6% ($p<0.05$). These findings reveal the protective role of fermented milk against H_2O_2 -induced skin aging though MAPK signaling pathway (Figure 6).

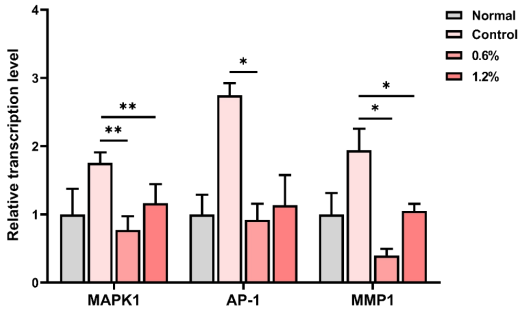


Figure 6. Fermented milk inhibited the increase of the transcription levels of the MARK and its downstream genes. *p<0.05, **p<0.01.

ROS can significantly activate ERK, and antioxidants can reduce ERK phosphorylation. To further validate the inhibitory effects of the fermented milk on the MAPK signaling pathway in response to oxidative stress, the expression of ERK and phosphorylated ERK (p-ERK) was examined in HaCaT cells. As shown in Figure 7, the p-ERK/ERK ratio of SFML pretreatment was significantly lower than that of H_2O_2-induced control group. It suggests that pre-incubation with 0.6% SFML before H_2O_2 exposure prevents ERK from being phosphorylated. These results further confirm that SFML can protect HaCaT cells from ROS damage by inhibiting the MAPK pathway.

(**a**) (**b**)

Figure 7. The protein content of the ERK and p-ERK in HaCaT cells pre-incubated with 0.6%、1.2% SFML. The control group was treated only with H_2O_2, while the normal group was treated with neither SFML nor H_2O_2. Grayscale values are processed by Image J. *p<0.05.

3.7 Promotion Wound Healing

Cell migration plays a crucial role in wound healing. An in vitro scratch assay was conducted to measure the rate of gap closure in sheets of HaCaT cells after co-cultivation with CFFF and SFML, respectively. The results indicated that both CFFF and SFML promoted cell migration, resulting in almost gap closure within 24 hours, and the migration rates of HaCaT cells co-cultivation with CFFF and SFML were similar to the observation of the epidermal growth factor (EGF), suggesting the probiotic role of fermented milk in skin repair (Figure 8).

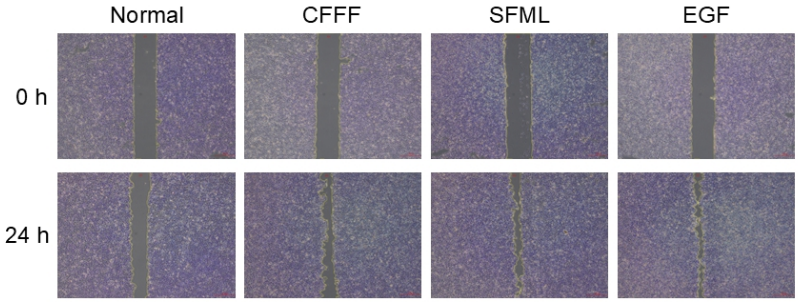


Figure 8. Effect of the CFFF and SFML on in vitro wound healing with HaCaT cells. Normal, no any treatment cells; CFFF, cells treated with cell-free fermented filtrate; SFML, cells treated with supernatant of sonicated fermented milk lysate; EGF, cells treated with epidermal growth factor.

4. Discussion

Lactic acid bacteria, as primarily probiotic strains, are widely acknowledged for their beneficial effects on both gastrointestinal and dermatological health[21]. This study demonstrated that SFML significantly promoted the expression levels of the tight junction proteins, upregulated the transcription levels of antioxidative enzyme genes including CAT, SOD, and GPX in HaCaT cells. Furthermore, co-incubated with SFML prior to H_2O_2 exposure effectively reduced the intracellular ROS content, inhibited the activation of the MAPK signaling pathway, downregulated the AP-1, MMP-1 gene transcription, and also prevented ERK from phosphorylation. Moreover, SFML promoted wound healing, thereby revealing the protective mechanism of the fermented milk by *S.thermophilus* CGMCC24468 against H_2O_2-induced damage, highlighting its potential application as natural ingredient in cosmetics products for preventing skin photoaging.

Compared to other lactic acid bacteria, a notable characteristic of *S.thermophilus* is its sophisticated defense systems against oxidative stress, enabling it to grow in the presence of oxygen and survive at low concentrations of H_2O_2[22]. This study showed that *S.thermophilus* CGMCC 24468 exhibited robust free radical scavenging capacity in vitro (Table 1), suggesting the attribution of the antioxidative enzyme SOD. Additionally, *S.thermophilus* also contains other antioxidative defense mechanisms such as NADH oxidase, Dye-Decolorizing Peroxidase EfeB, and antioxidative metabolites including glutathione, thioredoxin, as well as exopolysaccharides produced by *S.thermophilus*[23]. Therefore, *S.thermophilus* CGMCC24468 or its fermented products can serve as an effective antioxidant agent to elimination free radical. Moreover, the fermented milk by *S.thermophilus* CGMCC 24468 regulated the transcription levels of antioxidant enzymes (SOD, CAT, and GPX) in HaCaT cells prior to H_2O_2 exposure, thereby reducing intracellular ROS levels comparable to those observed in untreated HaCaT cells (Figure 5). This indicated that SFML activated the cellular antioxidant defenses and conferred strong antioxidative ability to keratinocytes, aligning with observations that the reported Lactobacillus species and Bifidobacteria have beneficial effects on skin health such as antioxidant, anti-aging, and repairing ROS damage[24].

MMP-1 is a collagenase, and the increase in MMP-1 levels leads to the degradation of collagen and elastin, leading to skin sagging and wrinkles[25]. In this study, an H_2O_2-induced damage model using HaCaT cells was employed. It was observed that H_2O_2 exposure significantly increased the transcription levels of the related MAPK signal pathway genes, including ERK gene and its downstream AP-1, MMP-1 genes in HaCaT cells, suggesting that H_2O_2 activated the transcription factor AP-1 via the ERK signaling pathway, thereby promoting MMP-1 expression which contributing to skin aging or photoaging. However, co-incubation with SFML prevented the upregulation of those genes, further confirming its cytoprotective role against ROS-induced damage. These findings were according with previous studies that lactic acid bacteria exerted antiphotoaging effects by eliminating free radicals, enhancing peroxidase activity, and inhibiting MMPs formation[26].

The presence of free amino acids, oligopeptides, vitamins, exopolysaccharides, and bioactive enzymes in *S.thermophilus*-fermented milk renders it an excellent nourishing source for both the skin and its microbiomes. Previous research was shown that the ingredients derived from *S.thermophilus* enhanced sphingomyelinase production, promoted skin hydration, and slowing the aging process[27]. Topical application of a cream with sonicated *S.thermophilus* lysate increased the moisture and ceramide levels in the stratum corneum of atopic dermatitis (AD) patients, indicating its positive role in maintaining skin integrity[28]. Therefore, *S.thermophilus* CGMCC 24468 fermented milk is a potential natural ingredient for cosmetic formulations, or skincare products.

5. Conclusion

In this study, the co-incubation of SFML and HaCaT cells upregulated the expression of antioxidant enzyme genes and reduced the oxidative byproduct ROS. It further inhibited ERK and MAPK phosphorylation, preventing the activation of the MAPK/ERK pathway, thereby reducing AP-1 and MMP-1, which in turn decreased the degradation and damage to the extracellular matrix and downstream active substances. Concurrently, the fermented milk also upregulated the expression of tight junction proteins, promoting cell migration and ensuring the stability of intercellular tight junction structures. This indicates that, in addition to its fermentation function, *Streptococcus thermophilus* has potential applications in skincare.

6. Reference

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