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Probiotic Improves Skin Oxidation, Elasticity, and Structural Properties in Aging Rats

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ABSTRACT: Skin aging, which affects all living organisms, is associated with oxidative stress. Probiotics exhibit antioxidant properties by producing reactive metabolites that counter oxidative stress. We hypothesized that *Limosilactobacillus fermentum* USM 4189 (LF 4189) has antioxidative properties and may prevent skin aging. In the present study, we used a D-galactose senescence-induced rat model to evaluate the potential antioxidative capability of LF 4189. The results indicated that rats administered LF 4189 exhibited increased plasma antioxidative activity (P=0.004), lipid peroxidation capacity (P=0.007), and skin elasticity compared with untreated aged rats (P=0.005). LF 4189 prevented telomere length shortening (P<0.05), indicating the potential to prevent senescence. A higher apoptotic activity was observed in old rats compared with young rats, whereas LF 4189 reduced the expression of four antioxidative enzyme genes that function as radical scavengers (all P<0.05), suggesting that the LF 4189 group had a reduced need to scavenge free radicals. Our findings indicate the potential of probiotics, such as LF 4189, as an anti-aging dietary intervention with antioxidant potential to improve skin health.

Keywords: aging, antioxidant, probiotic, skin, Sprague-Dawley rat

INTRODUCTION

The skin is the largest organ in the human body and aging is an inevitable and complex natural process. There are two types of aging processes that affect the skin: intrinsic aging and extrinsic aging. Intrinsic aging is primarily the result of genetic and hormonal influences, whereas extrinsic aging may be caused by environmental factors, such as UV light, pollution, infection, and toxins such as cigarette smoke (Kober and Bowe, 2015). Skin changes associated with aging include dullness, wrinkles, reduced moisture, thinning, loss of elasticity, and pigmentation disorders (Udompataikul et al., 2009).

Several factors contribute to skin aging and the molecular changes involved are complex. The underlying biochemical and molecular mechanisms include changes in pH, telomere shortening, oncogene activation, altered immune response, and oxidative stress, which can cause DNA damage resulting from the production of reactive oxygen species (ROS) (Udompataikul et al., 2009; Chen et al., 2016). Therefore, understanding these processes is essential for the development of effective anti-aging products and interventions.

Because aging is irreversible, maintaining a healthy life-

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style can delay or prevent the onset of age-related irregularities. Nutraceuticals such as dietary supplements have become popular because of their ability to prevent or treat various diseases (Ong et al., 2022; Nisaa et al., 2023). Probiotics represent live microbial supplements that have beneficial properties to the host when administered in sufficient amounts (FAO, 2006). The demand for probiotic-containing foods has increased substantially worldwide as research continues to provide evidence for their potential health benefits.

Probiotics, which primarily consist of lactic acid bacteria, can modulate the composition of gut microbiota and the skin immune system, leading to the maintenance of homeostasis (Guéniche et al., 2009). Probiotics are also known for their high antioxidant and immunomodulatory properties as they regulate cytokine signaling pathways (Mohammadi et al., 2015). Oxidative stress is one factor associated with aging and an increase in inflammatory markers and maintaining a well-balanced gut microbiota can ameliorate the aging process (Kumar et al., 2016). Therefore, incorporating probiotics into the diet may be an effective way to prevent or delay the onset of age-related skin irregularities.

Previous studies have revealed the positive effects of probiotics on rats through *in vivo* experiments accompanied by biochemical assays. These include anti-aging experiments using a mouse model induced by D-galactose (D-gal) because of its resemblance to the normal aging process. D-gal produces a glycation by-product that is associated with aging and age-related disorders (Thakur et al., 2017), thus the D-gal-induced mouse is a reasonable skin-aging model. In the present study, we evaluated the anti-aging effects of a probiotic strain *Limosilactobacillus fermentum* USM 4189 (LF 4189) on skin aging using a D-gal-induced rat model.

MATERIALS AND METHODS

Bacteria strains and media

Stock cultures of LF 4189 were preserved in 20% glycerol at −20°C. Each strain was grown and activated in sterile de Man-Rogosa-Sharpe broth (Biomark) three successive times using 10% (v/v) inoculum and incubated at 37°C in an aerobic atmosphere without agitation prior to use.

Animal models and diets

All experimental procedures involving animals were conducted under Good Laboratory Practice conditions and facilities according to the National Institutes of Health Public Health Service Policy and approved by the Animal Ethics Committee USM [USM/Animal Ethics Approval/2016/(724)]. Eight-week-old male Sprague-Dawley rats were obtained from the BRIMS Animal Facility of Monash University Malaysia (Selangor, Malaysia). A standard laboratory chow diet and water were provided ad libitum. Following acclimatization for 1 week, the animals were divided into four groups (n=6; 3 rats/cage). Premature senescence induction in rats was done as previously described (Hor et al., 2019). The treatment groups were as follows: Young, naïve rats received 0.9% saline via subcutaneous injection daily; Old, rats received 600 mg/kg D-gal via subcutaneous injection to induce aging; Old+4189, aged rats treated with LF 4189 (1×10^{10} CFU/d) via oral administration; Old+metformin, aged rats treated with metformin (300 mg/kg/d) via oral administration. The rats were housed individually during feeding to ensure complete pellet consumption and sacrificed by carbon dioxide asphyxiation after 12 weeks. The skin from each rat was removed, collected in a microcentrifuge, and snap-frozen in liquid nitrogen. All samples were stored in −80°C until further analysis.

Ferric reducing antioxidant power assay

Measurement of the ferric reducing ability of plasma (FRAP) was performed as previously described (Benzie and Strain, 1996). Briefly, 10 μL of sample and 40 μL of water were combined in a microplate well. Then, 200 μL of working reagent (acetate buffer pH 3.6, $FeCl_3$ solution, 2,4,6-tripyridyl-s-triazine solution; 10:1:1) were added to each well and the mixture was incubated for 8 min at 37°C. The absorbance was measured at 600 nm using a Thermo Scientific Multiskan Go plate reader against a reagent blank (Thermo Scientific). $FeSO_4$ standards were to generate a calibration curve and the FRAP assay results were expressed in μM.

Malondialdehyde (MDA) assay

The MDA assay was performed using the thiobarbituric acid (TBA) method (Bhutia et al., 2011). Briefly, 0.5 mL of serum was mixed with 0.5 mL of 30% tricholoroacetic acid (Merck), centrifuged at 1,100 *g* for 5 min, and the supernatant was collected. Next, 0.5 mL of supernatant was incubated with 0.5 mL of 1% TBA (Merck) in boiling water bath for 30 min and the tubes were placed on ice for 10 min. The absorbance was measured at 532 nm against a blank reference. The MDA concentration was plotted based on a 1,1,3,3'-tetra-ethoxypropane (Merck) standard calibration curve and the lipid peroxidation results were expressed in μmol/L.

Tensile strength measurements

The tensile strength measurement of rat skin was performed using a micro-tensiometer as described previously. Briefly, skin specimens were placed into a Petri dish containing phosphate buffered saline (Gibco) and carefully punched through to obtain strips of a pre-determined size. The strips were then transferred to a Petri dish con-

taining phosphate buffered saline for tensile testing. The tensile strength test was done using an eXpert microtester (Admet, Inc.) with a 10 N load cell in static mode.

Skin histology

The dorsal skin of the rats was carefully harvested to form an exposure area of 4×5 cm^2 in size. The skin samples were fixed in neutral-buffered formalin, trimmed, and a dehydration and clearing process was carried out using an automatic tissue processor (Shandon Excelsior, Thermo Scientific). The samples were embedded in paraffin wax and positioned perpendicular to the cut surface in tissue blocks. The blocks were serially sectioned into 4 μm-thick sections and stained with hematoxylin and eosin dyes prior to histological examination.

Measurement of telomere length

Genomic DNA was extracted from whole blood collected by heart puncture. Telomere length was measured using quantitative polymerase-chain reaction method as previously described (Cawthon, 2009). Briefly, 20 ng of DNA sample was added to SensiFAST SYBR mix (Bioline) along with the telomere and single copy gene primers listed in Table 1.

Measurement of gene expression

Rat skin was stored in RNAlater (Ambion) at −80°C. Total RNA was extracted by homogenizing the samples in TRI Reagent® (Sigma-Aldrich) and first-strand cDNA was synthesized using the ReverTra Ace-α-® kit (Toyobo). Gene expression was measured using an Agilent AriaMX Realtime PCR System (Agilent Technologies). Briefly, 20 ng of cDNA was combined with SensiFAST SYBR mix and 10 μM of the following primers: cyclin D1, FAS, glutathione peroxidase (GPX), and superoxide dismutase (SOD) (Table 1). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an endogenous control.

Statistical analysis

Data were analyzed using IBM SPSS Statistics 22.0 (IBM Corp.) software and the results were expressed as the mean±standard error of the mean. Differences among experimental groups were analyzed using a one-way ANOVA with *post hoc* analysis using Tukey's test. Independent *t*-tests were used to compare between treatment groups. Two-way repeated measure ANOVA was used for time-based experiments. $P<0.05$ was considered statistically significant.

RESULTS

Antioxidant potential

The plasma of old rats exhibited a lower antioxidative potential compared with that of young rats, which was determined using a ferric reducing antioxidant activity assay ($P=0.017$; Fig. 1A). However, treatment with LF 4189 significantly prevented this reduction ($P=0.004$) to a level comparable with that of old rats without treatment (Fig. 1A). Lipid peroxidation, which was determined using concentrations of MDA, indicated a significantly higher level in old rats compared with young rats ($P=0.020$; Fig. 1B). Following treatment with LF 4189, a significant decrease ($P=0.007$) in MDA levels in old rats was evident (Fig. 1B). The trend in rats treated with LF 4189 in both experiments was similar to those treated with the control drug metformin. These findings suggest that the administration of LF 4189 enhanced the antioxidant capacity of blood in rats, thereby preventing an increase in MDA levels.

Skin elasticity

Tensile strength measurements revealed that older rats did not show a significant loss in skin elasticity compared with younger rats, whereas the administration of LF 4189 to old rats significantly increased skin elasticity ($P=0.005$) compared with untreated old rats (Fig. 2). Similarly, rats in the positive control group that were treated with metformin also exhibited a similar effect ($P=0.002$).

Skin histology

Fig. 3 shows four photomicrographs of skin sections from

Table 1. Primer sequences for gene expression analysis

Primers	Forward 5′-3′	Reverse 5′-3′
Cyclin D1	GTGAGGAGCAGAAGTGCGAAGA	GGCCGGATAGAGTTGTCAGTGTAG
FAS	ATGAGATCGAGCACAACAGC	TTAAAGCTTGACACGGACCA
GPX	CACAGTCCACCGTGTATGCC	AAGTTGGGCTCGAACCCACC
SCG	CGGCGGCGGGCGGCGCGGGCTGGGCGGAAATGCTGCACAGAATCCTTG	GCCCGGCCCGCCGCGCCCGTCCCGCCGGAAAAGCATGGTCGCCTGTT
SOD	ATGGCGACGAAGGCCGTGTG	GACCACCAGTGTGCGGCCAA
Telomere	ACACTAAGGTTTGGGTTTGGGTTTGGGTTTGGGTTAGTGT	TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAACA
GAPDH	CCATCCCAGACCCCATAAC	GCAGCGAACTTTATTGATGG

GPX, glutathione peroxidase; SCG, single copy gene; SOD, superoxide dismutase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

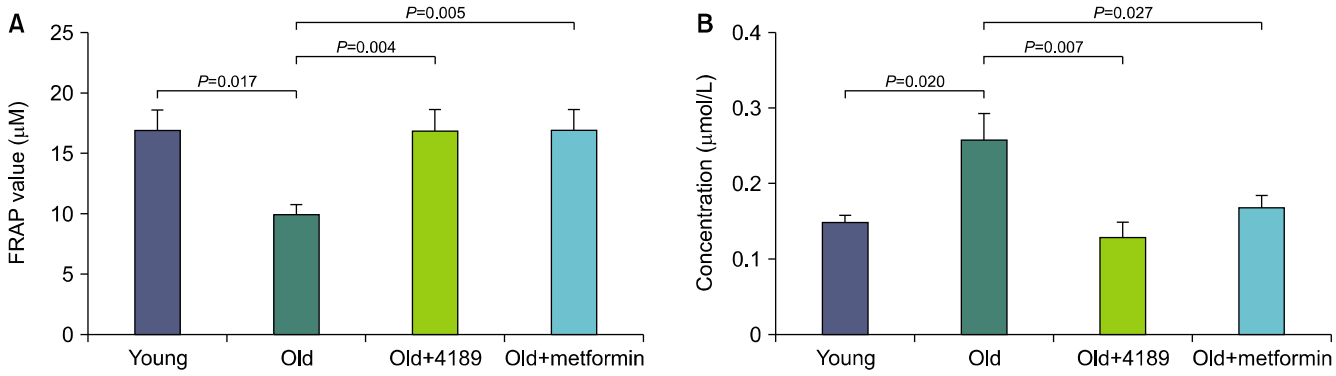

Fig. 1. Plasma antioxidant potential as determined by (A) ferric reducing antioxidant power (FRAP) and (B) lipid peroxidation marker via concentrations of malondialdehyde following 12 weeks of treatment. Treatment groups include: Young, naïve rats receiving 0.9% saline via subcutaneous injection daily; Old, rats receiving 600 mg/kg D-galactose via subcutaneous injection to induce aging; Old+4189, aged rats treated with *Limosilactobacillus fermentum* USM 4189 (1×10^{10} CFU/d) via oral administration; Old+metformin, aged rats treated with metformin (300 mg/kg/d) via oral administration. Results are expressed as the mean±SE; n=6. Statistical analysis was conducted using an independent *t*-test.

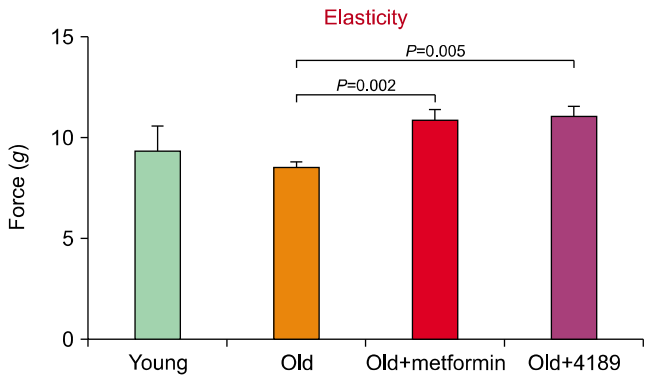

Fig. 2. Skin elasticity as measured using a micro-tensiometer following 12 weeks of treatment. Treatment groups include: Young, naïve rats receiving 0.9% saline via subcutaneous injection daily; Old, rats receiving 600 mg/kg D-galactose via subcutaneous injection to induce aging; Old+4189, aged rats treated with *Limosilactobacillus fermentum* USM 4189 (1×10^{10} CFU/d) via oral administration; Old+metformin, aged rats treated with metformin (300 mg/kg/d) via oral administration. Results are expressed as the mean±SE; n=6. Statistical analysis was conducted using an independent *t*-test.

a young rat (Fig. 3A), an old rat (Fig. 3B), a metformin-treated old rat (Fig. 3C), and an old rat treated with LF 4189 (Fig. 3D). Photomicrograph A and B indicate the normal histological features of skin, in which the skin is composed of the epidermis and dermis. The epidermal layer, which is composed of stratified squamous epithelium, was thicker in the young rats compared with old rats. The stratum papillare and stratum reticulare of the dermal layer could not be clearly distinguished in old rats, whereas it was composed of dense irregular connective tissue with collagen fibers and fibroblasts. The dermis of the old rats had a lower abundance of collagen fibers with more empty spaces compared with young rats, which indicated ground substance. The stratum papillare of the dermis of the young rats extended into the epidermis as dermal papillae, which was less evident in the old rats. The dermis of both groups contained skin appendages, including hair follicles, sebaceous glands, sweat glands, and sweat ducts, but nerve endings were not visible in both sections. Aging reduced dermal thickness (Fig. 3E), whereas the administration of LF 4189 prevented such a loss, which showed similar results with that of rats consuming metformin. Meanwhile, this effect was not observed in epidermis of rats administered metformin.

The subcutaneous tissue, a layer beneath the dermis, in both young and adult rats contains stratum adiposum and panniculus carnosus. The stratum adiposum contains adipocytes intervening through a looser structure of collagen fibers compared with the dermis, and this layer was thicker in old rats compared with that in young rats. The stratum adiposum is separated from the panniculus carnosus, a thin layer of skeletal muscle, by interstitial connective tissue. The subcutaneous tissue of the metformin-treated old rats contained scanty adipocytes separated by dense irregular connective tissue as in the dermis, with a less prominent panniculus carnosus. In contrast, the subcutaneous tissue of the LF 4189-treated old rats was more prominent, with a saw-tooth appearance of the stratum adiposum, and thicker panniculus carnosus. These findings indicate that treatment with LF 4189 improves the skin structure and elasticity of old rats.

In contrast to normal old rats, the skin sections of old rats treated with metformin and LF 4189 revealed a thicker dermal layer, which was reduced upon aging compared with young rats (Fig. 3E). The dermal layer of both metformin- and LF 4189-treated old rats exhibited a clearer demarcation of the stratum papillare and stratum reticulare with prominent dermal papillae, thus resembling the features of the skin of young rats. The dermis of both metformin- and LF 4189-treated groups contained more collagen fibers, fibroblast, and skin appendages compared with the skin of untreated old rats.

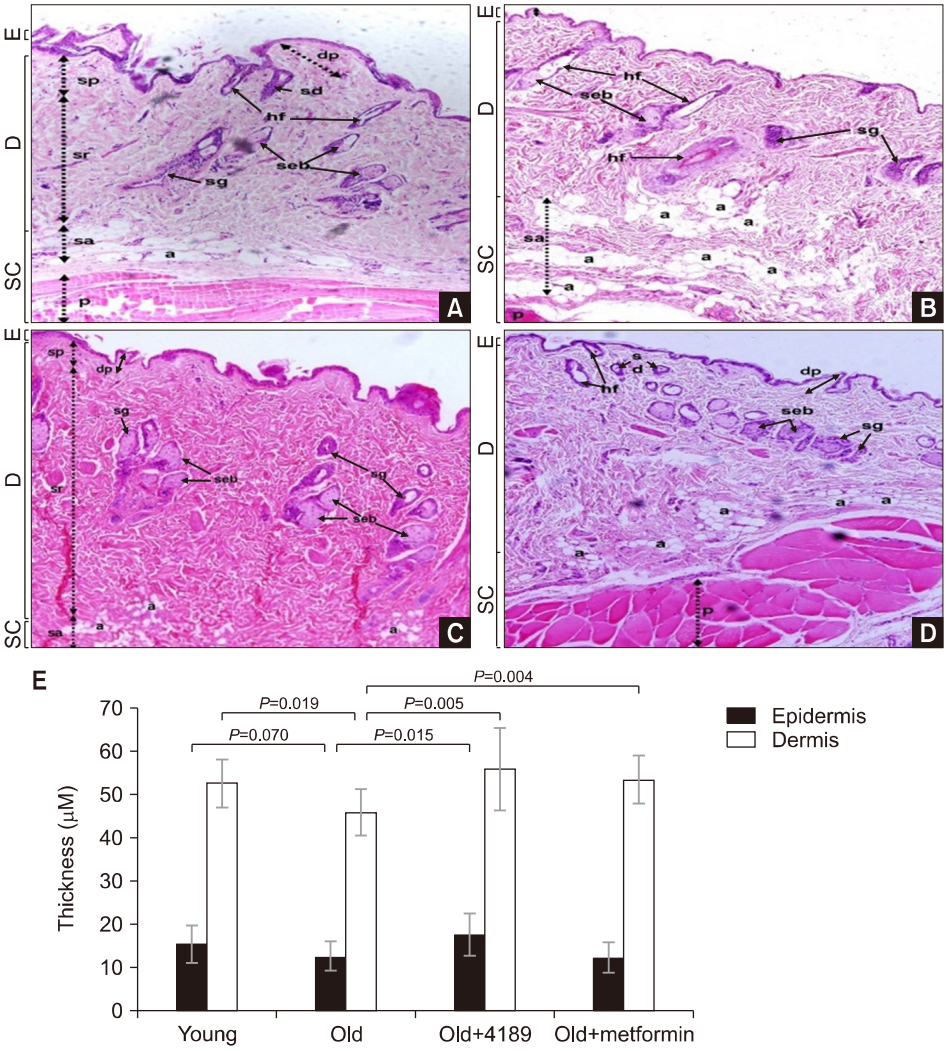

Fig. 3. Photomicrographs of skin sections from (A) young rat (naïve rats receiving 0.9% saline via subcutaneous injection daily), (B) old rat (rats receiving 600 mg/kg D-galactose via subcutaneous injection to induce aging), (C) old rat treated with metformin [aged rats treated with metformin (300 mg/kg/d) via oral administration], and (D) old rat administered LF 4189 [aged rats treated with *Limosilactobacillus fermentum* USM 4189 (1×10^{10} CFU/d) via oral administration]. E, epidermis; D, dermis; SC, subcutaneous tissue; sp, stratum papillare; sr, stratum reticulare; sa, stratum adiposum; dp, dermal papilla; a, adipose tissue; p, panniculus carnosus; hf, hair follicle; seb, sebaceous gland; sg, sweat gland; sd, sweat duct. (E) Thickness of the skin epidermis (■) and dermis (□) from the different treatment groups.

Telomere length

Old rats exhibited a shorter telomere length compared with young rats ($P<0.05$); however, old rats treated with LF 4189 prevented such a shortening compared with old rats without intervention ($P<0.05$), whereas metformin, as a positive control, did not exhibit such an effect (Fig. 4).

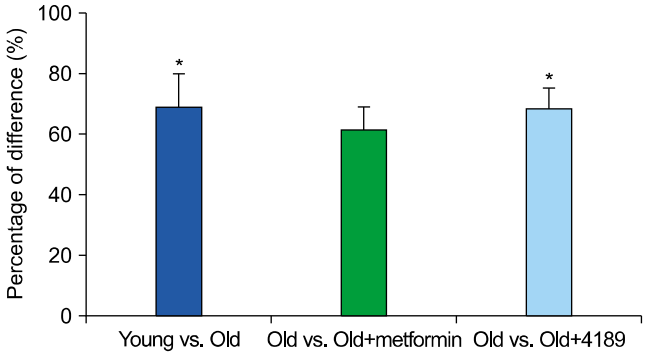

Fig. 4. Telomere length as measured by quantitative polymerase-chain reaction using genomic DNA. Young, naïve rats receiving 0.9% saline via subcutaneous injection daily; Old, rats receiving 600 mg/kg D-galactose via subcutaneous injection to induce aging; Old+4189, aged rats treated with *Limosilactobacillus fermentum* USM 4189 (1×10^{10} CFU/d) via oral administration; Old+metformin, aged rats treated with metformin (300 mg/kg/d) via oral administration. The results are expressed as the mean±SE; n=6. Statistical analysis was conducted using an independent *t*-test. **P*<0.05.

Gene expression

The level of the apoptotic marker *FAS* was significantly decreased ($P=0.004$) in old rats compared with young rats, suggesting a lower apoptotic activity present in the skin of old rats. The administration of LF 4189 to old rats significantly prevented the reduction of *FAS* expression ($P=0.035$) compared with old rats (Fig. 5A). The senescence marker *cyclin D1* was significantly increased in old rats compared with young rats ($P=0.004$). The administration of LF 4189 significantly prevented the increase of *cyclin D1* expression ($P=0.046$), suggesting reduced skin senescence (Fig. 5B). Expression of the oxidative stress marker *GPX* was increased in old rats compared with young rats, indicating higher oxidative stress in old rats.

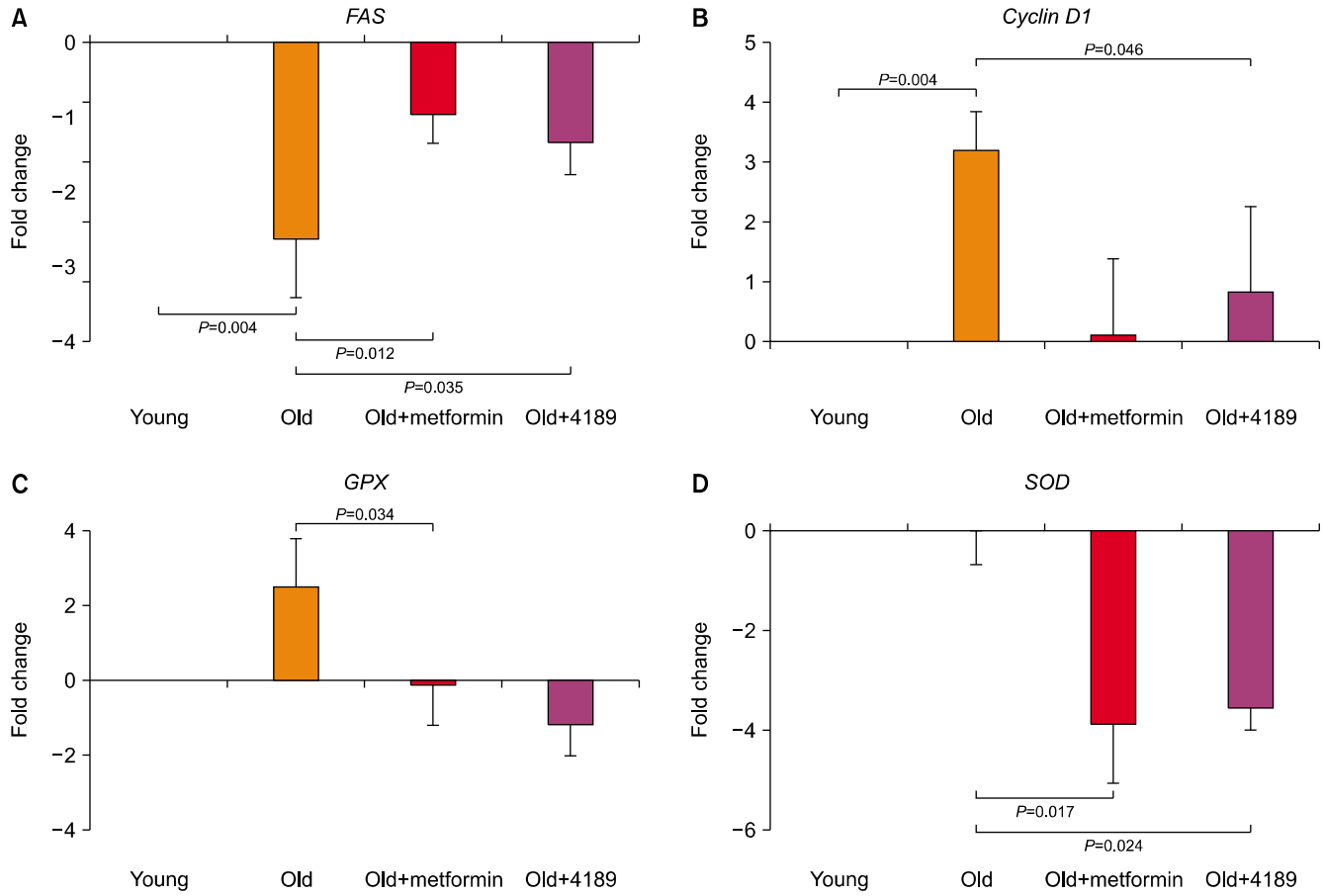

Fig. 5. Fold-change of gene expression levels for molecular markers involved in (A) apoptosis as determined using the *FAS* gene, (B) senescence as determined using the *cyclin D1* gene, and oxidative stress using (C) the *glutathione peroxidase* (*GPX*) gene and (D) *superoxide dismutase* (*SOD*) gene following 12 weeks of treatment. Treatment groups include: Young, naïve rats receiving 0.9% saline via subcutaneous injection daily; Old, rats receiving 600 mg/kg D-galactose via subcutaneous injection to induce aging; Old+4189, aged rats treated with *Limosilactobacillus fermentum* USM 4189 (1×10^{10} CFU/d) via oral administration; Old+metformin, aged rats treated with metformin (300 mg/kg/d) via oral administration. The results are expressed as the mean±SE; n=6. Statistical analysis was conducted using an independent *t*-test.

Following the administration of LF 4189, *GPX* (P=0.034) and the antioxidative marker *SOD* (P=0.024) were significantly decreased (Fig. 5C and 5D, respectively), indicating lower oxidative stress and a reduced the need to defend against oxidative stress.

DISCUSSION

Skin possesses better and more efficient antioxidant activity compared with other tissues in the body (Kohen and Gati, 2000). This activity reduces with age and is an important factor that results in skin aging. Oxidative stress is widely considered to be a major contributor to aging and age-related diseases (Liguori et al., 2018). Living organisms require oxygen for survival and free radicals are produced as natural by-products of cellular metabolism, which leads to DNA damage, membrane peroxidation, and functional group modification (Ray et al., 2012). Blood plays a central role in maintaining redox balance as it transports and distributes antioxidants throughout the body. Thus, evaluating blood oxidative stress levels is important (Katalinić et al., 2007). In the present study, total plasma antioxidant capacity was measured using the FRAP assay. The results indicated that plasma antioxidant capacity in old rats was significantly lower compared with that in young rats. Conversely, the administration of LF 4189 to old rats increased their antioxidant capacity to an extent comparable with that of young rats. Lipid peroxidation was evaluated based on plasma MDA levels based on the susceptibility of membrane lipids to free radicals (Ayala et al., 2014). Our results indicated that MDA levels in the plasma of old rats increased, whereas they decreased after administering LF 4189 to old rats. MDA levels increased with age and the extent of lipid peroxidation also was dependent upon age. This indicates that antioxidant capacity decreases with age and LF 4189 could buffer such a detrimental effect. While many physiological mechanisms may have occurred, our data indicate that LF 4189 exhibits an antioxidative effect through one of the mechanisms involving increased FRAP values and lower MDA levels.

Skin aging is characterized by various changes, including the decline in cell division or lipid levels in keratinocytes, a decrease in spinous cells, decrease in collagen fibers, thinning of fiber bundles, and a decrease in elastic fibers in the dermis (Hori et al., 2010). These changes are responsible for the loss of normal elasticity during aging. Our results showed that the skin of old rats exhibited lower elasticity compared with that of young rats; however, the administration of LF 4189 prevented the loss of elasticity during aging. Histological imaging revealed that the administration of LF 4189 to aged rats contributed to a significant increase in epidermal density and composition, accompanied by a clear demarcation of the stratum papillare and stratum reticulare layers with prominent dermal papillae, more collagen fibers, fibroblasts, and skin appendages, as well as a more prominent subcutaneous tissue with a saw-tooth appearance of the stratum adiposum and thicker panniculus carnosus, compared with untreated old rats. These factors are important as it shows that LF 4189 enhances skin elasticity and provides protection against environmental stressors, ultimately reducing the appearance of fine lines and wrinkles. The clear demarcation of the stratum papillare and stratum reticulare layers with prominent dermal papillae contribute to improved skin structure and provide support and organization to the underlying layers, which effectively reduces sagging skin. Collagen is the main structural protein of the skin and plays an important role in maintaining its structure and elasticity (Thornton, 2013). Fibroblasts are responsible for the production of collagen and other extracellular matrix components. An increase in the number of collagen fibers and fibroblasts in the skin in our present study was associated with improved skin elasticity and provided more support and firmness to the skin (Oh et al., 2022). Moreover, skin appendages, such as hair follicles, sweat glands, and sebaceous glands, play an essential role in maintaining skin health and function. An increased number of skin appendages often results in improved skin structure and elasticity, which is attributed to better skin hydration, oil production, and protection from environmental stressors (Zhang et al., 2023). Finally, the subcutaneous tissue, which is the layer of fat beneath the skin, provides insulation, cushioning, and energy storage. In the present study, the observed increase in subcutaneous tissue exhibiting a saw-tooth appearance of stratum adiposum and thicker panniculus carnosus contributed to improved skin health by providing enhanced support and cushioning to the skin, while reducing sagging skin (Velasco et al., 2022). Oxidative stress caused by an imbalance between the production of free radicals and their neutralization results in the breakdown of collagen and elastin fibers in the skin, and subsequently, the structural integrity and elasticity of the skin. Antioxidants help counteract the effects of oxidative stress by neutralizing free radicals and reducing potential damage to the skin. We hypothesize that LF 4189 improves skin elasticity during aging and is associated with antioxidative potential.

Telomere shortening is associated with cellular aging and certain diseases (Holohan et al., 2014). In a rat model induced by D-gal to mimic the aging process, galactose production through biochemical processes resulted in the accumulation of galactitol in cells due to aldose reductase catalysis, which ultimately resulted in cell swelling, dysfunction, and aging (Thakur et al., 2017). A comparison between young and old rats in the present study revealed a significant difference in telomere length, with shorter telomeres in older rats. This indicated that telomere length decreases during aging. The administration of LF 4189 to old rats resulted in a significant difference in telomere length compared with old rats without intervention, similar to old rats administered metformin, a positive control that showed improved telomere length in a previous study (Hor et al., 2019). As D-gal metabolism may contribute to the generation of ROS through oxidative metabolism, which could result in aging, the effects of ROS may be mediated through telomerase-associated cell senescence (Buckingham and Klingelhutz, 2011). The telomere, which is composed of TTAGGG repeats, is sensitive to oxidative stress because of the G-rich region that is prone to base alterations by ROS (Kawanishi and Oikawa, 2004). Considering that probiotics exhibit antioxidative properties, we hypothesize that this is beneficial against telomere shortening and skin aging.

It is important to replace damaged cells with healthy cells because of the damaging effects of oxidative stress. Apoptosis, a type of programmed cell death, replaces damaged cells with healthy ones and plays a vital role in maintaining a healthy cell population during development and aging (Elmore, 2007). Activating specific genes in the apoptotic signaling pathway can achieve this; however, in certain circumstances, not all cells undergo apoptosis. Cells that receive overwhelming stress undergo apoptosis, whereas those that sustain less severe damage undergo senescence (Vousden and Lane, 2007). A reduction in *FAS* expression, an apoptosis marker, was significantly higher in old rats than young rats, indicating that older rats cannot regenerate healthy skin cells through the *FAS*-mediated signaling pathway. In contrast, after LF 4189 was administered to old rats, a reduction in *FAS* expression was prevented, indicating skin regeneration through the removal of old skin cells via apoptosis. The increase in ROS regulates *FAS*-stimulated apoptosis in several studies (Sinha et al., 2013; Tan et al., 2016).

The aging process is associated with cellular senescence and contributes to the inability to maintain tissue homeostasis and oxidative stress is a contributing factor (Sasaki et al., 2014). Senescent cells remain metabolically active

and undergo cell cycle arrest at the G1/S phase. The expression of *cyclin D1*, an important regulator of the G1/S transition that determines cell proliferation was significantly higher in old rats compared with young rats (Fernández-Hernández et al., 2013); however, the results indicated that old rats treated with LF 4189 exhibit a significant decrease in *cyclin D1* expression.

The response to oxidative stress is important for maintaining cellular homeostasis and the antioxidant enzyme system increases the antioxidative effect by scavenging free radicals (Kurutas, 2016). An important antioxidant enzyme is SOD, which catalyses the breakdown of superoxide into water and hydrogen peroxide. The glutathione (GSH) functions to counteract hydrogen peroxide, lipid hydroperoxides, xenobiotics as a cofactor of enzymes (Pole et al., 2016). GPX is a cofactor enzyme for GSH that converts hydrogen peroxide to water and attenuates lipid peroxidation (Rinnerthaler et al., 2015). The expression of both *SOD* and *GPX* were reduced in old rats treated with LF 4189, indicating a lower need to resist oxidative stress. The LF 4189 probiotic exhibited sufficient antioxidative effects to scavenge ROS as evidenced by the high antioxidative properties of old rats treated with LF 4189 in accordance with the FRAP assay. It has been previously reported that the antioxidative capability of probiotics are effective in disease-related conditions as evidenced by increased erythrocyte SOD and GPX activities as well as total antioxidant status (Ejtahed et al., 2012).

In conclusion, the administration of LF 4189 to older rats with high oxidative stress improved skin health during the aging process, primarily by preventing the loss of elasticity and skin structure. The probiotic also enhanced antioxidant capabilities and reduced the need to activate antioxidative enzymes against oxidative stress. Our findings indicate that probiotics represent a promising next-generation therapy for improving skin health during aging.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: SNHH, YHP, MTL. Analysis and interpretation: BKL, PX, UMM, MTL. Data collection: BKL, EIEH, KMN. Writing the article: BKL, SNHH, MTL. Critical revision of the article: WSJ, SDT, GL. Final approval of the article: all authors. Statistical analysis: BKL, MTL. Obtained funding: MTL. Overall responsibility: MTL.

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